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GLIAL CELLS

A Summary of an NRP Work Session
held June 2-3, 1964

Chaired
by

Robert Galambos

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Dedicated to the memory of our late colleague Charles M. Pomerat who ignored considerable discomfort to give this meeting the benefit of what turned out to be his last scientific testament.

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INTRODUCTION

The NRP Work Session on "Glial Cells" was held on June 2-3, 1964 at the NRP Center in Brookline, Massachusetts.

Its main purpose was to accumulate a state-of-the-art survey, hopefully somewhat ahead of the glia literature, on the following topics:

I. MECHANICAL PROPERTIES OF GLIA

(Pomerat, Altman, Bornstein)

II. GLIAL ENZYMES

(Friede, Barrnett)

III. GLIAL ELECTRICAL ACTIVITY

(Ranck, Tasaki and Walker, Adey)

IV. MYELINATION, DEMYELINATION AND MEMORY

(Bornstein, Akert, Randt, Williams)

Several important problems in glia physiology were deliberately excluded from consideration with the thought that these would receive full treatment at another time. Most conspicuously absent in this account is a discussion of memory and altered RNA base ratios in glial cells.

Our coverage will be found incomplete even on matters we deliberately undertook to cover. Thus, we devoted a large effort to the distribution of enzymes in glial cells but time limitations permitted Dr. Barrnett to discuss in detail only one enzyme, an ATPase, although his own techniques permit identification of some 25 different ones in the brain. He and Dr. Friede, who has studied the distribution of 6 enzymes in formalin-fixed material, provided most of our data from their own experiments, making no pretence of doing justice to the results of many others working on the same and related problems.

It was agreed that the enzyme repertoire of glia varies widely, and that a glia classification on the basis of their enzyme composition would be valid and useful. How best to classify the glial cells was a recurrent question and

despite general dissatisfaction with the purely morphological classifications currently accepted (Fig. 1), it was felt that we had neither the time nor the necessary information to undertake a revision at this time.

Whether glia cells are concerned with memory was a central theme for the session. The conferees repeatedly referred to those properties of glia that might be related to memory, and some interesting hypotheses were advanced. As might be expected, however, no final answers emerged. Glia proliferate with use, transport materials to the neurons, regulate the ionic environment of the brain, reorganize their enzymic content as needed, and respond with movements, electrical activity and impedence change to chemical and electrical stimuli. Through such activities glial cells could control or regulate neuronal firing in the formation and retrieval of memories, but no evidence favoring such a view is entirely convincing.

From such considerations as these, we reaffirmed our conviction that glia is a kind of tissue in its own right, with special functions and properties experiments continue to describe and define. These special properties, however, unless related to those of the neurons, fail to take into account the intimate symbiotic relationship between glia and neurons that is so widespread in brain and peripheral nerve. This symbiosis by itself implies for glia a role that cannot be discovered or studied by looking at glia alone. Glia in nature do surround neurons, and if this is so in order to prevent neurons from losing some material important for their function (as was suggested during the conference), no one could discover this until he studied the two cell types, neurons and glia, in their natural condition.

A final word is in order regarding Dr. Pomerat's contribution. His comments were reduced into semifinal form by the NRP staff and brought into its final form by the chairman of the session, who takes all responsibility for any errors that may be found in it. This paper represents Dr. Pomerat's last contribution to science, being followed within two weeks by his untimely death. The enthusiasm, vigor and clarity of his presentation will always be remembered by those privileged to hear it. Students of the brain, and in particular those with special interest in the function of glial cells, have lost from their ranks one of their most sophisticated and devoted members.

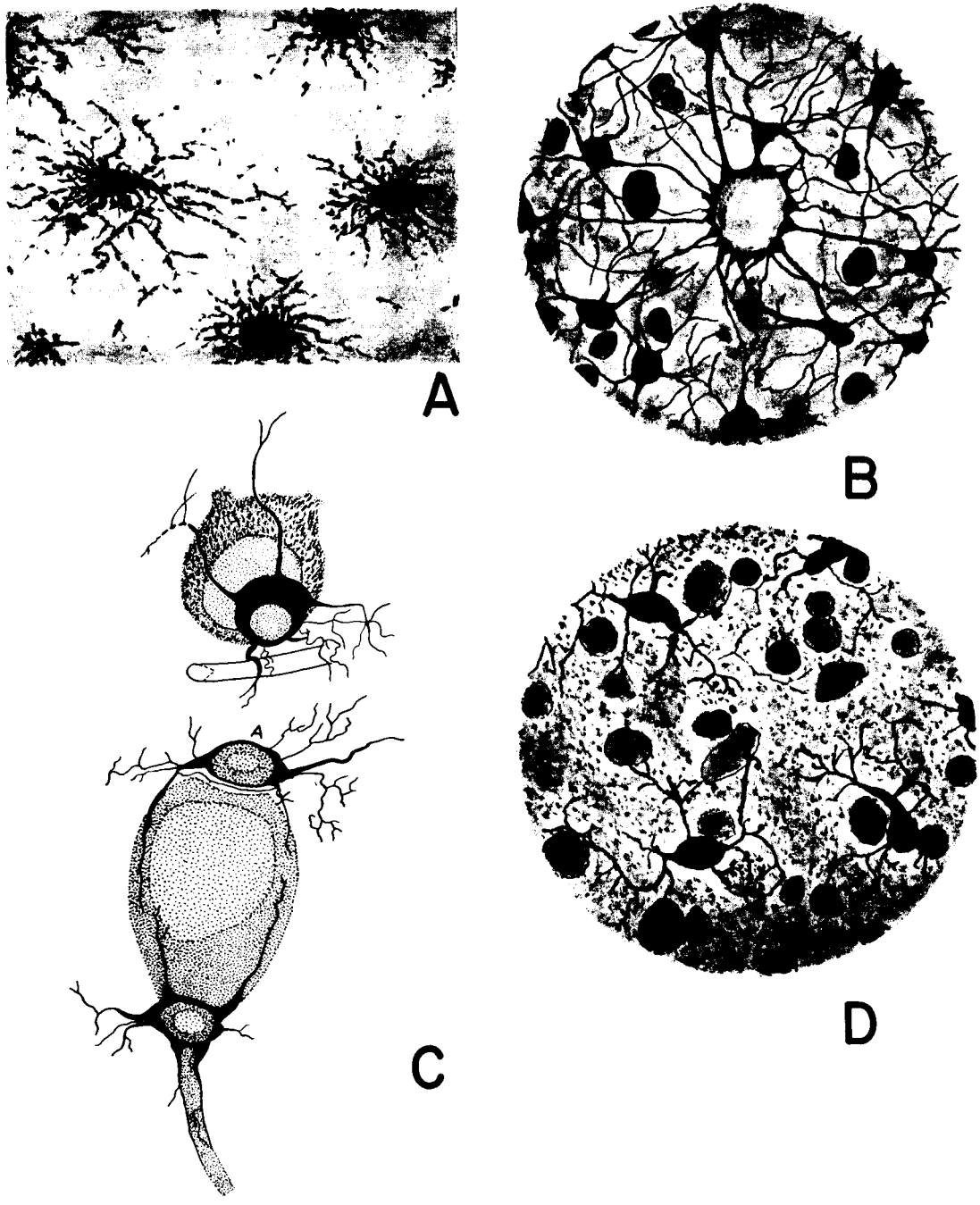

Figure 1. Traditional morphological types of glial cells. A: protoplasmic astrocytes, B: fibrous astrocytes with end feet around small blood vessel, C: oligodendroglia in close proximity to neurons, D: microglia. Pictures from Glees, P. (1955): Neuroglia Morphology and Function. Springfield, Ill. Thomas,C.

I. MECHANICAL PROPERTIES OF GLIA

A. Glial Motility in Vitro

Dr. Pomerat showed moving pictures of typical nerve and glial cells in tissue culture, emphasizing in particular the points discussed separately below. His technique involves time-lapse photography applied to cells visualized by phase contrast optics at very high magnification; the pictures show extensive movement of cell boundaries and cytoplasmic contents, with recognizable differences in activity between the various cell types. An inference to be drawn from the pictures -- that such activity takes place in the normal brain also -- is unsupported by direct evidence, but if this should be the case, the functions brain performs would have to take place in spite of (or because of?) considerable mechanical motion of its components.

1. Glia and Axoplasmic Flow

Pomerat suggested that a relationship may exist between the movement of material along axons (neuronal axoplasmic flow) observed by Weiss[33] and the glial rhythmic activity observed by himself. Axons can be several meters long, and if, as seems likely, material is actually transported all the way from the region of the cell nucleus (perikaryon) to the tip of such long axons, a responsible force or forces must be postulated. If the glia that surround the axon along its course should contract, these movements could exert pressures of various sorts, "massage" the nerve fiber, and thus provide forces to move materials along its length.

a. Rhythmic Contraction of Glia

Rhythmic contraction is particularly conspicuous in oligodendroglia and Schwann cells. Normal oligodendrocytes (from rat corpus callosum, in the movies) exhibit pulsatile movements: the cell processes alternately grow thicker and thinner, while the cytoplasm moves alternately between the perinuclear zone and the processes. The contraction-relaxation cycle takes 5 to 10 minutes. Schwann cells in culture also show similar movements, and it is mainly from this evidence that Pomerat suspected that normal Schwann cell contractions in peripheral nerve are responsible for the axonal peristaltic wave.

b. Movements within Developing Neurons

In the movie showing neurons in embryonic chick dorsal root ganglion, mechanical activity of various kinds appeared. Neuronal nuclei rotate (on the average of one complete rotation in 80 minutes, especially in regenerating neurons and for as long as 58 hours); peristaltic waves in axons are seen -- continuous undulatory movement accompanied by changes in outline observed continuously during a three month period; some granules move rapidly along nerve fibers (25 microns in 14 seconds) while mitochondria do so slowly; there are plastic changes in membranes at boutons terminaux, and pinocytosis occurs at the end of growth cones. Growth cones of two types can be identified, the one with filipodia, the other without; Pomerat suggested possibly different functions (sensory vs. motor?) for these restlessly moving tips of growing neurons.

c. Discussion by Weiss

After complimenting Dr. Pomerat on the technical quality of the photography, Dr. Weiss stated that, in his own similar material, mature axons have a definite peristaltic rhythm of 16 $\pm$ a few minutes per pulse, and this is not synchronized with pulsations of the Schwann cell. In his opinion the Schwann cell supplies either energy or substrate for energy to the axon, which has few mitochondria.

He cautioned that conclusions about mature fibers *in situ* should not be drawn from cultures as immature as Pomerat's. Neurons engaged in "frustrated regeneration attempts," *i.e.*, ones with neither dendrite nor axon connected to another cell, behave differently from mature connected fibers.

On the question of whether cell movements *in vitro* were comparable to those *in vivo*, Weiss proposed a theory that any incompressible fluid body enclosed by a semipermeable lipid-protein membrane, when actuated locally by any excitation, will enter into rhythmic peristaltic or pressure-wave pulsations, because of the local permeability changes, due to dilations across the phospholipid layers followed by local contraction of the unit membrane. Weiss described resultant surface pulsations in slime molds, kidney cells, and other cultured cells *in vitro*, and fish eggs, illustrating this type of self-perpetuating beat.

2. Schwann Cell Motion and Myelination

Peterson and Murray at Columbia as well as Pomerat's group at Pasadena have seen Schwann cell nuclei circumnavigate myelinating nerve fibers in culture. Pomerat's example rotated through 360 degrees in 44 hours, the total number of turns seen being one and a half. Pomerat suggested that this movement is intimately related to myelinogenesis, and raised the question of whether the CNS counterpart of the Schwann cell, the oligodendrocyte, acted similarly.

3. Function of Oligodendrocytes

Pomerat cited the suggestions of de Robertis and Bennett that glial foot processes may be embedded in axoplasm and recalled Cammermeyer's idea[5] that some oligodendroglia may regulate vascular flow. He then pointed out that oligodendrocytes are the most numerous cell in the brain and suggested an analogy between brain physiology and immunology, in each of which cases one finds a ubiquitous cell (oligodendroglia and lymphocyte respectively). Does the oligodendrocyte carry information in the brain as does the lymphocyte in the immunologic case? (If antigen is injected into the toe pad of a rabbit whose lymph nodes are removed quickly thereafter, lymphocytes fail to respond to subsequently injected antigen with antibody; but if the lymph nodes are removed somewhat later, it is impossible to prevent them from doing so.) The significance of the fact that the oligodendrocyte is an RNA-rich cell is not known, but the RNA-richness may mean that experiential encoding occurs in glia.

Pomerat reported development of a cadmium sulfide scanner for automatically measuring the "diastole" and "systole" of glial pulsations. He hoped with this to investigate the effect of a variety of environmental factors on the contractile activity of the olidogendroglia. In the line of our interests, he suggested comparative in vitro studies of the motility and other responses of cells from brains of trained and untrained animals, or of cells from the two hemispheres of a split-brain preparation in which the memory was stored in only one hemisphere.

4. Discussion of Glia-Neuron Symbiosis

Bornstein, having noticed a dead neuron at a distance from satellite cells in Pomerat's movie, stated that in his own tissue cultures, whenever a neuron fell to the edge of the

explant any farther away from glia than three microns, that neuron invariably died. He has concluded that neurons probably need glia present within the usual neuroepithelial distances of Angstroms in order to survive, and wondered if contact between membranes and submembranous structures was necessary. Pomerat did not have proof, but he seriously doubted that neurons could survive without satellite cells. Weiss proposed a general principle of symbiosis between neurons and glia according to which neither does well in vitro without the other. The neuron is more active and breaks down sooner without auxiliary cells; but glia and Schwann cells, though they live a little longer, do not do well indefinitely. However, Ranck and Barrnett raised the exception that coelenterates have nervous systems that react to the same psychopharmaca as do higher nervous systems, yet they have no apparent satellite cells at all.

B. Glial Cell Multiplication in Vivo

Altman spoke on the multiplication of glial cells in vivo as observed by fine-resolution autoradiography. In his experiments, tritiated thymidine, a specific precursor of DNA synthesis, is injected, and the CNS tissue is then examined by microscope from animals after various post-injection survival times. By following the time course of label uptake in different areas of the brain, Altman has found that ependymal and subependymal cells divide, migrate to other areas of the brain, and differentiate into other types of cells in postnatal animals.

Although division, migration, and differentiation of cells from the ependymal region in embryos is well established as the mechanism of formation of the nervous system, Altman's observations in adult animals are unique. He has experimental evidence that these glia migrate to brain lesion sites in adult rats and differentiate into other types of glia, and that they participate in postnatal neurogenesis in young rats.

Since Altman found more glial cells of all types multiplying than he expected in normal adult animals, he is conducting experiments to test if glial multiplication is related to behavioral factors. Defining "learning" as the modification of behavior by experience, he has divided littermates into two groups whose life experiences were restricted to "impoverished" and "rich" environments and is analyzing whether the glial population differs in the two

groups. The results of these experiments were not available at the time of the Work Session.*

1. Glial Response to Lesions

When needle stereotaxic lesions were produced in the lateral geniculate body of rats, and the thymidine injected by the same needle, labeled glial nuclei were found along the entire visual pathway from the lateral geniculate body to the striate cortex.

The timing and the form of the label were puzzling. The earliest-seen labeled cells looked like oligodendroglia and were located in the cortical radiation, while the label did not reach the lesion area until one to two weeks later and the cells looked like astrocytes and normal and phagocytic microglia. The most plausible interpretation Altman could find for this was that the earlier-appearing neuroglia transformed into the later-appearing microglia. Bornstein reinforced this interpretation since he has seen neuroglia transform into phagocytic form in his tissue cultures.

When Altman published his experiment he thought that the glial proliferation would follow the degenerating pathway, so he described the occurrence of labeled cells in fibrous pathways -- corpus callosum, non-visual cortical structures -- as anomalous. However, later findings removed both the anomaly and the dengerating-pathway idea. Ependymal and subependymal cells were found proliferating along the ventricular wall, particularly the lateral ventricle. If the ventricle wall were to serve as a source of multiplying cells, migration of these cells along fibrous pathways would explain both the timing and the sites of the label. Altman's present interpretation is that, as a consequence of the lesion, multiplication of the ependymal and subependymal cells was induced and the cells then migrated to where they were needed, and in the process transformed from a relatively undifferentiated form to distinct types of glia. This hypothesis was partially confirmed by determining that for a unilateral lesion a larger number of cells were labeled in the dorsal roof of the lateral ventricle on the lesion side than on the normal side.

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* "Note: Increased rate of glial multiplication was obtained in the cortex of the 'enriched' animals. Results published in Nature (Altman and Das, 1964; in Bibliography)." (J.A.)

Although Altman sees mitotic figures in dividing glial cells of very young animals, he never sees them in adult cells. Instead he sees cells in a form seemingly pulling apart. To establish that the label was a direct measure of cell divisions, counts were made of the number of labeled cells per homologous brain area in animals of different survival time. The decrease in label concentration with time, as the number of labeled cells increased, proved to be reasonably explained by cell divisions.

2. Normal Adult Animals

In normal rats, systemic injection of label revealed dividing cells of all types: ependymal, perineuronal, and intrafascicular glia (see Fig. 2). This was generally true for all animals observed from 2 weeks to 8 months of age.

3. Postnatal Neurogenesis

While studying glial multiplication during development, Altman was surprised to discover labeled neurons. At the age of 10 days, 20 per cent of the animals' granule cells were labeled in the dentate gyrus of the hippocampus. Although this would suggest a high rate of cell division, mitotic figures were rarely seen and the label uptake was later found to decrease much too rapidly with age to be explained by cell division. Again migrating cells provided the answer. The appearance of labeled granule cell neurons correlated with the disappearance in the dentate gyrus of small glia-like cells. The appearance of these cells in turn correlated with the disappearance of mitotic cells in the lateral ventrical, with a sharp peak at 15 days. Examination of the brains of young normal rats revealed a layer of ependymal and subependymal cells, obviously embryonic and full of mitotic cells, following a pathway to the hippocampus. This layer gets increasingly smaller up to the age of thirty days, but more anteriorly one can see a source of mitotic cells that may survive in the adult animal; and experiments are continuing on this.

4. Discussion

a. Hippocampus

Since the granular cell area of the hippocampus is the area where the EEG theta rhythm is best developed, Adey wondered if Altman's neurogenetic observation explained Adey's

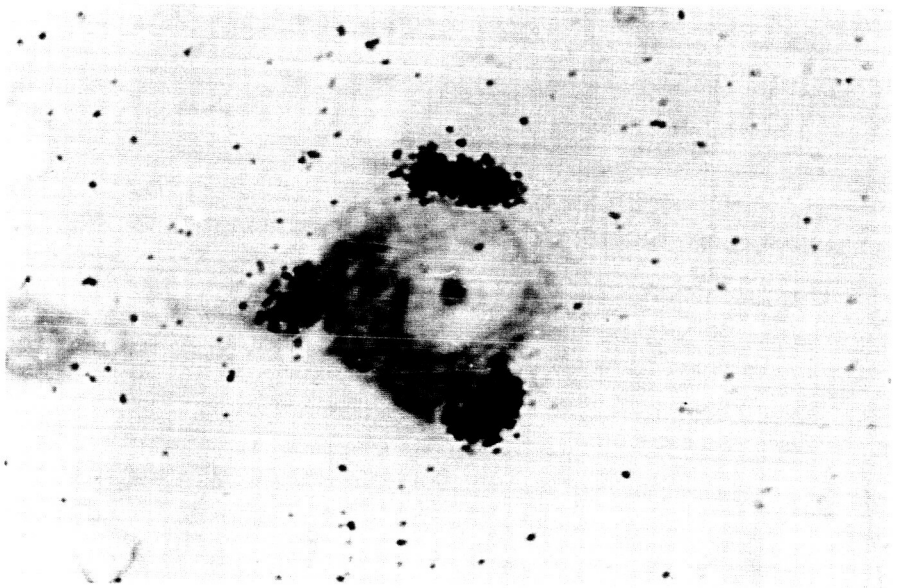


Figure 2. Rat spinal cord neuron showing three labeled perineuronal glia which came into existence at age 3-4 months in a normal rat. The small dots are "noise."

finding that the appearance of the theta is delayed in rats and kittens.

Altman considers that the delayed formation of the hippocampus may be related to delayed sexuality in animals. When tritiated sex hormones were injected into castrated and ovariectomized rats, the hippocampus granule and pyramidal cells were the only cells in the CNS that took up the hormone.

b. Glial Turnover versus Multiplication

Ranck raised the possibility of an antiglial theory of memory: that, instead of multiplying, the glia may be turning over, and that the turnover rate may depend on the rate of destruction of glia. Altman expects to have a conclusive experimental answer to glial destruction within a year, but a tritiated leucine study on glial multiplication in the optic tract of unilaterally enucleated pigeon conclusively showed that that process was multiplication. Furthermore, the label uptake of the glia was almost totally nuclear rather than cytoplasmic, implying that the increase in protein activity on the lesion side was also associated with DNA metabolism and cell multiplication. He also observed that the glial nucleus took up more leucine when the cell gave the appearance of multiplying. Finally, Galambos pointed out that it is well established that during development glia multiply, and that in aging the glia remain while neurons drop out.

c. Peripheral Nervous System

Considering that the size of motor ganglion cells varies with the peripheral load, Weiss raised the question whether the number of satellite glia cells also increased, and suggested that Altman's technique would be an excellent way to find out. Friede noted that Kuhlenkampf[19] observed twelve years ago that the number of motoneuron satellite cells increased with increased motor activity, but the question was open whether the increase was due to cell migration or to local cell multiplication; and again Altman's technique was suggested as a good means to get the answer.

d. Numbers and Location of Glia Relative to Neurons

Although the numerical ratio of glia to neurons ("glial index") is phylogenetically correlated only with size of brain, the glial count for man is high; and the significance of this

was explored: Why does the number of glia in man remain the same while neurons are lost with increasing age? Why, during human development, do massive numbers of glia appear around the apical dendrites of the cortex? Barrnett said that he rarely found fewer than a half dozen glial processes around a neuron soma, but since electron microscopy is a two-dimensional process the number of processes cannot be related to the number of glial soma. Altman, however, has seen areas of rat cortex without glial soma. Bornstein noted that during maturation of the neocortex the packing density of the neurons decreases, and he can see glial processes interposing themselves between neurons in tissue culture.

Akert described relevant observations of glia in the ant brain. In the highest area of the ant cortex, the mushroom body, the outer layer of densely-packed neurons is arranged so that eight to ten neuron somata are wrapped by processes from one glial cell. Neuronal fibers project downward from the cell layer, and synapses are extracortical (but have not yet been studied in relation to glial cells). This raises the question of a correlation between number of glial cells and number of synapses: Do the glial cells add some specificity to soma membranes? And would this explain why higher animals have more synapses at the soma? In support of this, Ranck cited a study by Bodian,[3] where in the spinal cord of monkeys, the somata were found half covered with glia and half with synapses whereas the more distal dendrites were almost totally covered by synapses, with few glia to be found.

Altman cited Flechsig's work published in 1876,[9] where he divided the areas of the cortex into association and projection types, and claimed that the myelination in the association areas continued up to 20 years of age. Altman wondered if this had anything to do with the glial index.

e. Need for Operational Redefinition of Glia

Bornstein raised the point that 19th century staining terminology does a disservice to current techniques. Instead of talking about "astrocytes" and "oligodendroglia," we should also make functional differentiations on the basis of modern methods of analysis. There may be profound differences, for instance, between subependymal cells, cells forming myelin, satellites to neuron somata, purely structural elements, cells with one foot on a capillary, etc.

f. Glia - Intercellular Space - Dementia

Adey cited work by Harry Zimmerman[29,30] at Montefiore Hospital in New York with colleagues at Yeshiva University, where in both senile and presenile dementias and in Alzheimer's Disease, a sponginess was found in the brain. Specifically, this sponginess consisted of an increase in the volume of the glial fraction in the gray matter, and an increase in extracellular fluid in the subcortical white matter. This certainly raises the question of the relationship of glia to extracellular space, and the relation of both to dementia.

II. DISTRIBUTION OF ENZYMES IN GLIA

A. By Light Microscopy

Friede is attempting to differentiate glia from each other on the basis of enzymatic activity. By staining CNS tissue for oxidative and glycolytic enzymes and counterstaining with histochemical stains, he hopes to replace morphological characterizations of glia with functional "job descriptions." The tissue, mostly human, is examined at the light microscope level.

At the Work Session, Friede discussed glia in the inverse order of the relevance he thought they may have to memory: 1) microglia, considered least relevant, function mainly in pathologic states as scavengers of dead or dying tissue; 2) astrocytes probably act to keep the ionic environment of neurons constant; 3) oligodendrocytes are the most likely candidates for a role in memory since a number of enzymatic relationships between oligodendrocytes and neurons exist, which indicate symbiosis between the two.

1. Microglia

It is generally accepted that microglia become particularly prominent in pathology, and Friede provided definite enzymatic evidence for this. Normally microglia stain very lightly for both oxidative and hydrolytic enzymes, but when they are proliferating in response to a pathological condition they stain more intensely for acid phosphatase than do any other glial cells under any condition; oxidative enzymes do not increase proportionally.

2. Astrocytes

Friede looked for a clue to the function of astrocytes in normal tissue by examining their characteristic reaction to hypertrophy in pathological tissue. The normal astrocyte stains so lightly for all oxidative enzymes studied that sometimes a counterstain is necessary to see the cells at all, while much activity occurs in neurons and neuropil. However, almost universally in pathologic tissue, the hypertrophied "reactive astrocyte" stains oxidatively more intensely than normal neurons ever do in the cortex, while other types of glia in the section do not increase in oxidative enzymes. In view of recent interest in the role of glia in maintaining the ionic balance of the CNS, Friede studied the effects of sodium concentration in tissue culture. While at a sodium concentration slightly lower than physiological the astrocytes were unaffected, at a higher concentration the astrocytes assumed a form and an increase in oxidative enzymes that looked exactly like the reactive form often seen in brain tissue. Since high sodium concentration can trigger the enzyme changes in tissue culture, Friede thought it reasonable that the astrocytes function *in vivo* to provide a constant ionic environment for neurons.

3. Oligodendrocytes

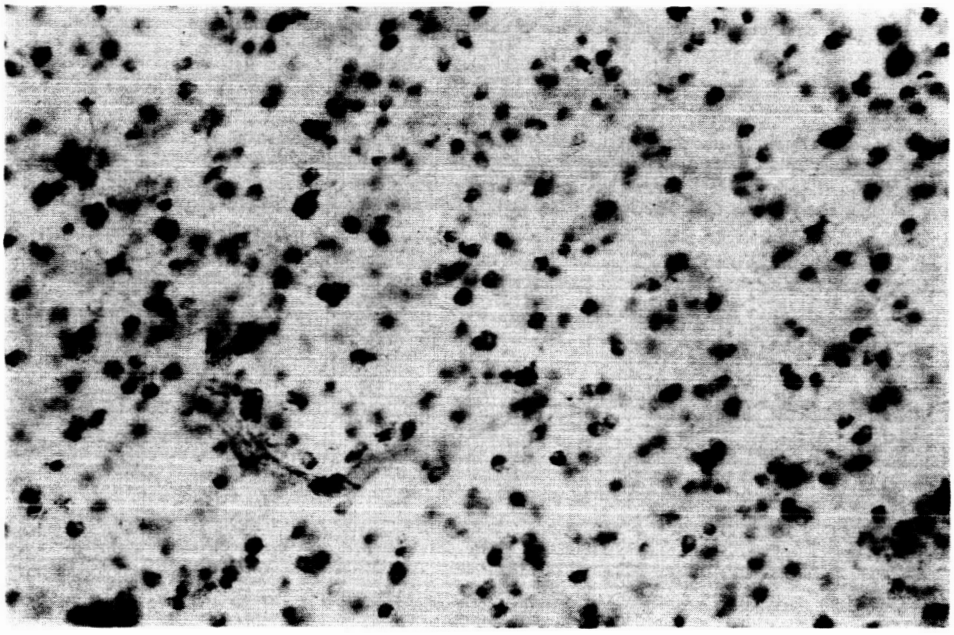
Friede described several enzymatic correlations between oligodendrocytes and neurons.

He cited relevant work of others indicating that the *ratios* of enzyme activity in white and gray matter differ for enzymes involved in energy metabolism: the ratio is roughly 1 to 2.5 for glucose-6-phosphate dehydrogenase (Lowry, *et al.*,[22] Robins and Smith[26]) but is larger for various glycolytic enzymes, and is largest -- about 1 to 8 for cytochrome oxidase (Pope[14,24]). The conclusion that these ratios reflect properties of oligodendroglia in white matter (Pope) is supported histochemically by showing that the staining in oligodendroglial cells for the respective enzymes varies according to the properties described above: it is most intense for glucose-6-phosphate dehydrogenase and least intense for cytochrome oxidase. Thus Friede agrees with Hydén that in the brain, oligodendrocytes exhibit more glycolytic and less oxidative activity than do neuronal perikarya and dendrites, though oligodendrocytes have more oxidative activity than do normal astrocytes.

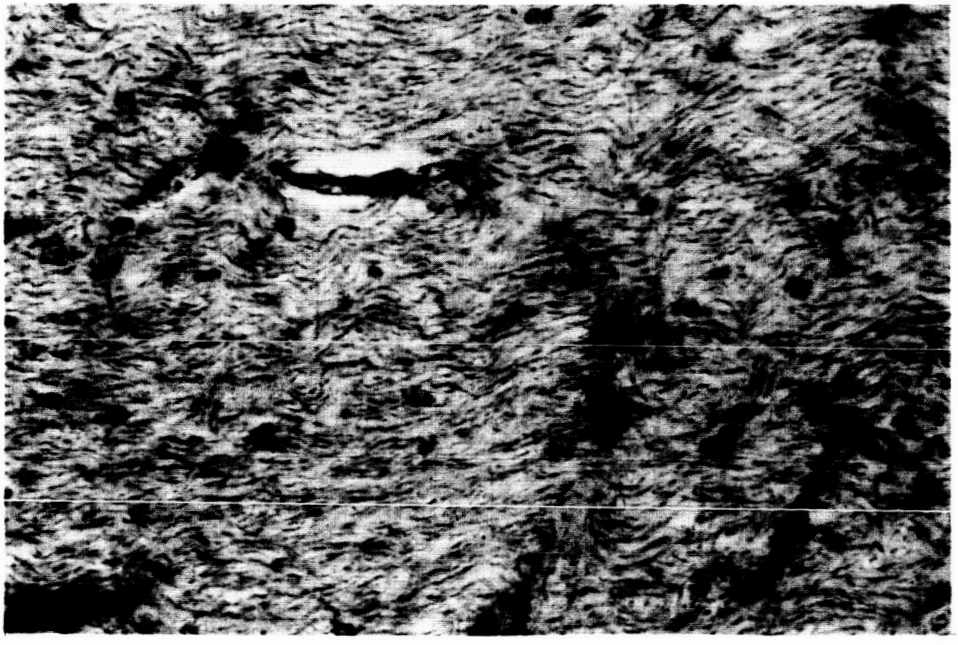
Staining for DPN-diaphorase is in white matter almost equivalent to a histochemical stain for oligodendrocytes. Using this stain, Friede has been able to distinguish between Hortega's morphological Types 3, 4, and 1 plus 2.(16) In this case he sees no functional significance to the distinction between 3 and 4. He cannot distinguish between 1 and 2; but 1 and 2 stain less than do 3 and 4. The differences between these types may either imply a functional difference or merely an adaptation to fiber size.

In all nerve tracts that Friede has studied so far, he finds an inverse relationship between the oxidative enzymatic activity (lactic dehydrogenase; DPN diaphorase) of axons and their fascicular glia; and he would suggest that the enzymatic activity of the glia is not rigid, but dependent on a symbiosis with the axons. Korey and Orchen(18) estimated that axoplasm contributes about one third of the total respiration of corpus callosum. Friede feels that this percentage varies among tracts, sometimes even within tracts, and also with functional conditions. He quoted the following observations: 1) He found instances where larger numbers of enzymatically active glial cells were associated with more weakly active axons (e.g., hemispheres), while other tracts show much activity in axons and few active glial cells (e.g., pyramidal tract)(see Fig. 3). 2) In the spinal column, various oxidative enzymes increase in activity along the course of the axons in the terminal portion of the tract. This increase in axonal activity is accompanied by a decrease of glial activity -- along the same tract. 3) At the time of myelination, the number of glial cells and the oxidative enzyme activity in them increase in all tracts. In some tracts, glial numbers remain at these levels after myelination (parietal and temporal white matter), but the number of glia decreases in the pyramidal tract after myelination. Friede though it significant that the axonal enzyme activity was much higher in the pyramidal than in the other two cases.

Some years ago Friede had published the suggestion that the higher an animal was phylogenetically, the higher was the "glial index," or the ratio of the number of glial cells to the number of neurons. Since that time, Hawkins and Olszewski(13) published that the glial index varied instead with the size of the different animals' brains, and this relationship seems to be the more fundamental statement. Friede has also found, in Clark's column of the spinal cord in the cow, that the longer the axon, the larger is the number of sattellite glial cells per unit area around the nerve cell.



Prevalence of DPN-diaphorase in glia
in white matter of human cerebral hemispheres



Prevalence of DPN-diaphorase in axons
in human spinal tracts

Figure 3

Also for different animals, the longer the average axon length of Purkinje cells -- as indicated by the size of the cerebellum -- the greater is the number of Bergman cells (perineuronal glia) per Purkinje cell.

4. Speculative Discussion

a. Memory

Friede suggested that the symbiosis he has observed may be a flexible and dynamic one. On increased demand, the neuron could respond by attaching more glial cells; and in this way glia may play a role in neuronal metabolism and possibly in memory as an auxiliary metabolic unit. This would be more reasonable than hypertrophy of neurons upon increased demand, which would ruin synapses.

b. Differentiation of Glia

Friede would suspect that all glia have an undifferentiated precursor; less differentiated glia might be more multipotential than higher differentiated types. Oligodendroglia, for example, do not seem to exist in lower species, apparently not below the level of the shark. It would be interesting to find out if in the lower animals the glia are multipotential, while in higher animals there is greater differentiation. This is of particular interest since Friede suspects that there is a spectrum of differentiation in the mature brain. He once observed Bergman cells, nominally astrocytes, to increase in enzymatic activity as reactive astrocytes do; as a rule, however, they do not show this response, raising the possibility that even in the human brain some glia are multipotential. Friede would include the Schwann cell of the peripheral nervous system as a member of this spectrum of multipotential differentiation since it serves alone in the peripheral nervous system as satellite cell to the same cranial nerve, that in the central nervous system has both astrocytes and oligodendrocytes as satellites. Functionally, therefore, glia are currently difficult to compare both phylogenetically and within any one animal.

Altman made the point that, although "short term" and "long term" memory are not yet precisely defined terms, that lower animals cannot remember for longer than about 2-3 hours. Maybe well-developed glia are important to long term memory.

c. Environmental Effects

Weiss emphasized that one cannot talk of cell "type" without identifying differential responses of the different cells to the same environments, including the responses of enzymes. This is an important unknown and could be explored in tissue culture.

B. By Electron Microscopy

1. Method

Barrnett reported on his electron microscopic studies that relate cellular enzyme activity to ultrastructure. He has worked with 25 enzymes and studied their relation to a number of cellular characteristics, but the one that has proven important in glia is nucleoside triphosphatase (ATPase), an enzyme widely implicated in active transport. He reported work with central nervous system tissue, including cerebrum and retinae, using his new technique of fixation with gluteraldehyde that preserves cellular organization but does not destroy enzyme activity. After subsequent incubation of the tissue with enzyme substrate, ATP in this case, and with lead ion, an electron-opaque product, lead phosphate, becomes observable at the site of enzyme activity.

2. Blood-Brain Barrier Hypothesis

Barrnett's findings on enzyme activity at astrocyte cell membranes, especially in relation to blood vessels, led him to a new concept of the mechanism of operation of the blood-brain barrier. Prior to this, he had found that throughout most organs of the body unfenestrated capillaries have ATPase-containing vesicles as pinocytic invaginations in the capillary wall but not in the endothelial plasma membrane; and he concluded that the mode of transport across such capillaries, previously a matter of controversy, was by pinocytic vesicles. This was also true of small vessels in parts of the brain outside the blood-brain barrier, such as choroid plexus and area postrema. Inside the blood-brain barrier, however, the capillaries were found to have very few vesicles and these contained very little or no ATPase. Instead, capillary walls were found to be "plastered with" astrocytic foot processes. These foot processes showed a localization of ATPase activity at the plasma membrane, and the contrast was striking between the strongly stained glial side of the capillary wall and the absence of reaction on the

endothelial side. There was occasional enzyme reaction on the basement membrane.

It was also a repeated finding in the central nervous system that glia-glia and glia-neuron interfaces always showed the same ATPase and neuron-neuron interfaces never reacted. The conclusion to which Barrnett came concerning the blood-brain barrier was that the barrier is due to the lack of transport enzyme in the endothelium of cerebral capillaries, and that transport of substances is facilitated by the basement membrane and glia, rather than barred, as is generally held. Only astrocytes are involved. Oligodendrocytes did not have ATPase activity. Barrnett believes therefore that astrocytes function in feeding substrate from blood vessels to neurons by active transport, as well as in regulating the ionic environment, the only function attributed to them by Friede.

3. Inbetween Cell Hypothesis

Barrnett found that when extracellular space was of the order of 120 A, ATPase was always found between glial and neuronal membranes and between the folded membranes of individual glial cells but never between neurons (see Fig. 4). He hypothesized that in tissues where extracellular space is limited, this space, or the membranes on each side of it, is the site of an enzyme functioning as a mechanism for the concentration of ions needed for cell functioning, at least active transport of Na and K. He extended this to say that the same type of enzymatic activity will be found wherever one cell separates another cell from extracellular space: the "inbetween" cell will have ATPase at its surface. Cases found by Barrnett outside the central nervous system include the Sertoli cells surrounding developing sperm, Schwann cells surrounding unmyelinated peripheral nerves, cells in the thymus where a blood barrier is known to exist, and free-floating leukocytes without surface enzymes, which suddenly acquire enzymes at the surface when they contact other cells.

4. Discussion

Schmitt questioned the idea of the biosynthetically-inferior astrocyte feeding the biosynthetically-superior neuron, and raised the question of whether glia instead act "in spite of" something. Altman pointed out that they could still be a barrier to passive transport.

Figure 4. Electron micrograph of rat retina showing dense staining of ATPase around all glial processes, but none between neurons.

Akert described a striking analog to vertebrate glia separating neurons from capillaries in ant brain glia which are interposed between tracheids and neurons. Processes from these glia converge at the end of tracheid arborization systems and then diverge to individual nerve cells. Similarly, Schmitt questioned nourishment as a role for these glia, and pointed out that there was no obvious need in a cold-blooded animal for interposition of a cell between the oxygen supply and the neuron, unless the glial cell were keeping something away from the neuron.

Schmitt also noted that ATPase is implicated in energy transduction as well as in active transport.

Bornstein added the suggestion that glia might also serve to keep something from leaving the neighborhood of the neuron, or from leaving the tight extracellular space. If memory should be related to an induction system, there must be some mechanism to maintain that state throughout the life of the animal. To postulate a role for the tight space, Bornstein mentioned 1945 experiments in which he showed that acetylcholine would tend to remain in intercellular space without being destroyed. This "free" acetylcholine might be available to re-affect neurons and to maintain an induced enzymatic alteration previously produced by the neuron's exposure to the direct stimulating (synaptic) effect of another neuron.

Schmitt suggested an analogy. Until about 10 years ago, it was thought that, except during cell division, the nucleus was a domain separate from the cellular cytoplasm. Now there is more support for considering the cell a biphasic system, where the DNA and the mechanisms for DNA readout (repressors and derepressors) are partitioned off from the non-coding, non-biosynthetic part of cytoplasm by the endoplasmatic membranes. As the nucleus is not really isolated from the "cytoplasm" so perhaps also the neuron is not really isolated from glial cells. Would it be profitable to seek electron microscopic evidence for direct, though transient, connections between neurons and glia across which coding as well as metabolic solutes might pass? Direct connections between micro-organisms are now known to permit coding determinants to pass from one micro-organism to another. Direct though transient connections between neurons and glia might provide a mechanism for the transport of molecularly encoded information, particularly at regions of neuronal nets, such as the synapse, where molecular switching may determine over which of the myriads of possible subnets impulses might travel.

III. ELECTRICAL CHARACTERISTICS OF GLIA

A. Specific Impedance of Cerebral Cortex at Different Frequencies

Ranck summarized his published experimental-theoretical results on ionic content and specific impedance of glia and interstitial space in vivo under normal conditions and in spreading depression (SD). For the Work Session he suggested an extrapolation to normal glial function of his interpretation of his spreading depression observations: that glia, presumably astrocytes, serve as a "potassium sponge" for neurons; and that the functional interrelation between glia and interstitial space involves changes in volume as well as ionic content.

1. Ionic Content

Ranck reconciled the small interstitial space discovered by electron microscopists with the large "sodium chloride space" formerly equated with the interstitial space. By comparative measurements of normal lateral geniculate tissue and tissue in which gliosis had been provoked, NaCl was calculated to be present in large amounts in glia, and the potassium was found to be as high in glia as in neurons. By doing an electric and osmotic balance, Ranck tentatively concluded that a significant amount of cation in glia exists in bound form.

2. Specific Impedance

Ranck studied the passive impedance characteristics across 1 mm. areas of cerebral cortex at frequencies of 5, 50, 500, 5,000, and 50,000 cycles/second. He followed both the amplitude, roughly equivalent to resistance, and phase angle, roughly equivalent to capacity and indicative of membrane effects. The characteristic result is that the impedance decreases with increasing frequency, and the phase angle is greatest at about 50 cycles per second and smallest at 5000 and 5 cycles per second.

He constructed a model to explain the data. Of particular interest is the phase angle change at 50 cycles/second. To explain it, he needed a longitudinal current in some fiber with a time constant of about 10 milliseconds, presumably neurons. Another cell with a membrane time constant of from 0.1 to 10 μsec. is needed. The experimental measurements by

Hild and Tasaki[15] provided the answer: glia. In general Ranck's model works with interstitial space of the size observed by electron microscopy. At distances longer than 5 microns, Ranck calculates from the "cable properties" that one-third of the glial cells function electrically as an effective interstitial space.

3. Spreading Depression

Ranck conducted similar impedance measurements on animals with spreading depression and interpreted the data in terms of his general model, to translate the impedance changes into changes in interstitial space and in cell membranes.

Since the impedance was independent of membrane characteristics at frequencies greater than 5000 cycles, large increases observed there must be due either to changes in the resistance of interstitial space or of intracellular cytoplasm. The magnitude of the changes seemed more reasonably due to interstitial space, and others have suggested that in SD the size of interstitial space decreases sizably.

An initial decrease in phase angle can only be explained as a decrease in the resistance of neuronal membranes, which is what would be expected since it is thought that potassium moves out into interstitial space in SD and depolarizes the neurons. The decrease in membrane resistance calculated from this was 1/7, a figure consistent with Araki and Terzuolo's finding for depolarized motoneurons.[1]

Of particular interest is a late increase in impedance and phase angle, while the general tendency was for membrane resistance to decrease. It can be shown because of the time relationships and other factors that the late increase cannot be due to neurons, but has to be due to an increase in membrane resistance of cells which initially had a low resistance -- that is, an increase in the membrane resistance of glial cells.

The mechanism that emerges from this is similar to "anomalous rectification," known to occur in heart and skeletal muscle. The increased membrane resistance of glia occurring late in the course of the SD may reflect a decrease in potassium conductance associated with a net outward driving force on potassium taking place at the time when the interstitial potassium concentration is decreasing. The net effect of this would be such that potassium enters the glial cells

more readily than it can leave.

4. Potassium Sponge Role for Glia

These experiments suggest that glia can take up potassium readily for short periods of time and then let it go more slowly over a longer period of time. If this were also possible under normal conditions, glia might act as a rate-meter for neuronal activity when potassium is released during action potentials and synaptic activity. The glia would prevent local build-ups in the concentration of potassium. And if cations are bound in glia, as the ionic studies suggested, this would imply further "sponging" ability.

Also implied here is that glia may modulate the size, as well as the ionic content, of interstitial space. Ranck would suggest that electron microscopists look for such variations in relative volumes of glia and interstitial space.

5. Discussion

Tasaki stated that the excitability of the neuronal membrane is unaffected by the potassium concentration within normal ranges of concentration. Ranck answered that Brinley, Kandel, and Marshall[4] estimated that the interstitial concentration of potassium in SD increases on the order of ten to twenty times, so that he feels his neuronal observations were valid.

Weiss pointed out that macromolecules are also present in interstitial space, so it can not be explained totally ionically. Recent experiments in his laboratory have shown that charged macromolecules microinjected into tendons travel preferentially and faster along the interfaces between the solid collagen fibers and their solid mucopolysaccharide matrix -- a phenomenon of "guided diffusion," which might generally prevail in narrow submicroscopic spaces in which free diffusion such as in ideal liquid solutions, is severely restrained.

B. Electrical Measurements: Single Cells

1. Microelectrode Measurements

Walker reviewed the work of Tasaki and Chang on the glia from the anesthetized cat brain, and that of Tasaki and

Hild on neuroglia from tissue culture which demonstrated that the resting potential of the glial cell could be reduced by electric shocks or the application of potassium chloride. This response was graded and exhibited summation thereby distinguishing the response of the neuroglia from the all-or-none response of neurons. The membrane resistance of the neuroglia was determined to be lower than that for neurons: 0.6-1.7 megohm or 3-10 ohm·cm^2.

Takenaka and Walker, working with Hild, repeated these measurements on ependymal cells and obtained results of the same order. They observed, as had Tasaki, that mesodermal cells from culture had a far less negative resting potential, which could not be reduced even by strong electric shocks. Adey commented that this was contrary to the experience of Wardell[32] of Oxford.

2. Pipette Measurements

Takenaka and Walker devised a method for measuring the impedance of glia obviating the use of intracellular microelectrodes. Minced cortical tissue from the brains of new-born animals was drawn into a pipette measuring 15 to 40 microns at its orifice and the impedance of the tissue measured. The mincing and the size of the pipette was designed to prevent the admission of living neurons. Tissue culture explants from midbrain and corpus callosum were also used.

Resistance of the tissue was measured as a function of the frequency of applied A.C. These measurements were rapidly made between 20 and 1000 cps to assure time-independent results. Tissue resistance was found to decrease from 20 to 200 cps, and thereafter to approach a constant value, which suggested that membrane resistivity could be measured only at A.C. frequencies below 200 cps.

3. Effect of Humoral Factors on Impedance

When KCl was applied to the tissue, the impedance of the glia dropped for 45 to 60 sec., followed by a sudden elevation in impedance lasting for 5 min. A D.C. shift was present during both phases of impedance change. The phase of elevated impedance was thought to represent a synchronous systole of the glia. A number of chemical agents, some of which are known to be present in the brain, affected the impedance measurement (see Fig. 5). Addition of calcium or serotonin caused an apparent cell systole; addition of cocaine

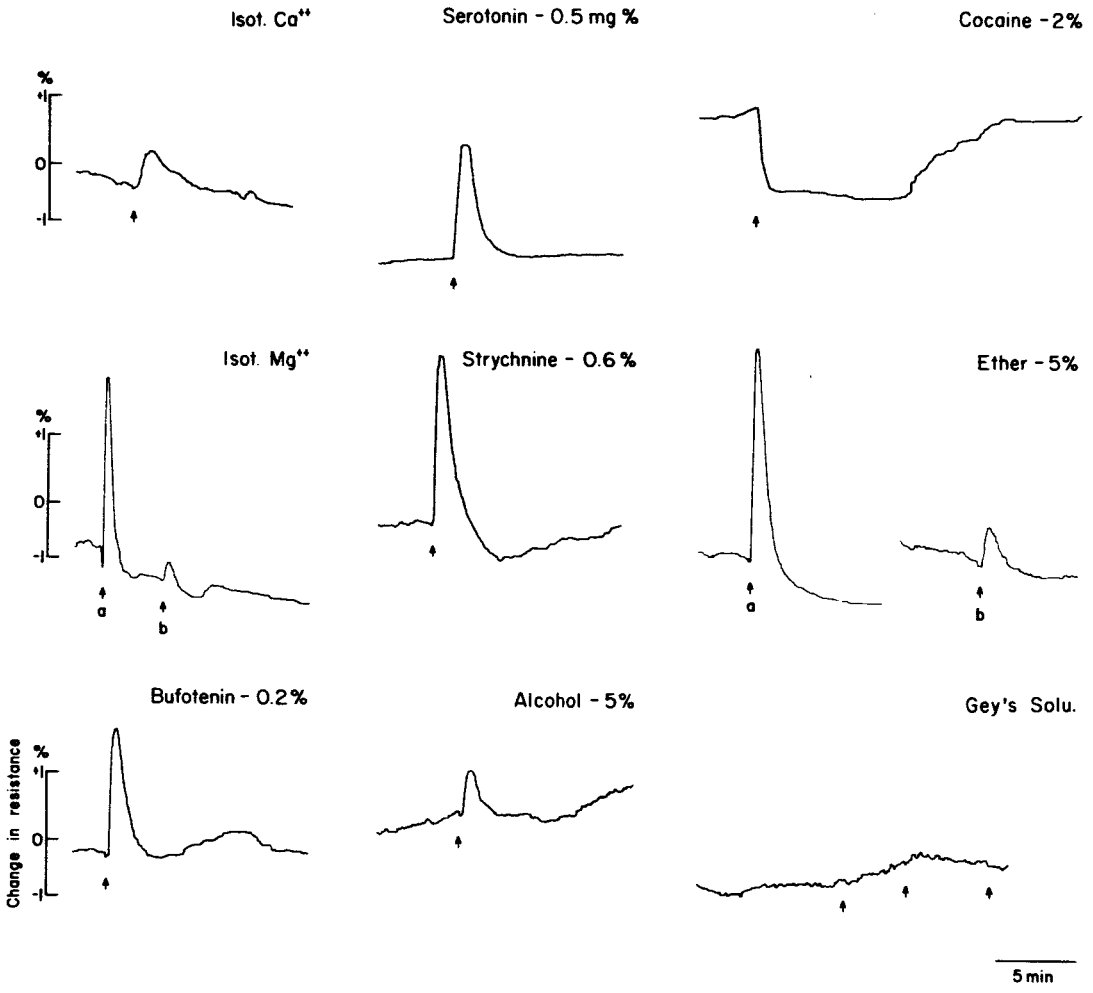


Figure 5. Effects of humoral factors on resistance of glia.

produced a decrease in membrane impedance. This led Walker to suggest that one of the major functions of glia is to serve as a receptor organ for humoral agents.

Galambos raised the question of whether glia in different regions of the brain have different properties with regard to what they will take up from the blood stream. Nauta suggested also regional differences in the glial-neuron relations. Adey cited his evidence of different sensitivity to carbon dioxide in different regions of the brain qualitatively and quantitatively, but he is uncertain that the effect is totally glial.

Friede, however, cautioned that in the areas where the evidence is strongest for receptor function, the relationship of neurons to blood vessels is the most intimate one, and there are relatively fewer glia present than in other brain regions. This re-opened the topic of whether glia function to keep something away from neurons. Altman recalled his experiment where labeled sex hormones reached only the hippocampus, and the dentate gyrus of the hippocampus has few glial cells.

4. Glial Response to Physical Stimuli

Using a smaller pipette, Walker found a measurable spontaneous change in impedance with time that followed much the same time course as Pomerat has shown for motion by light-sensitive cells. This activity was not seen in the larger pipette, probably because the larger number of cells cancelled out one another's activity. Weiss added that he has found a complete correspondence between electrical changes and contractile activity in the slime mold.

Akert believes that he has data indicating that ependymal cells in the subfornical organ of vertebrates are sensitive to infrared light. Galambos cited measurements[8] of isolated frog diencephalon where a peak in light sensitivity was obtained around 560 millimicrons; the actual receptors are apparently not identified. Schmitt thought that this was very important in that it might provide a means of signaling by modulation, providing there were appropriate receptors. It is well known that action spectra can be produced by injection of photodynamic dyes. Perhaps we should look into the possible role of the IR as well as the visible and ultraviolet spectral ranges.

C. Impedance, EEG: Focal Volumes

Adey is simultaneously evaluating the impedance and EEG of brain structures implicated in memory. By the use of computer techniques he can find subtle regularities in EEG's and shifts in impedance that are indicative of natural physiological processes. By positioning the electrodes across laminar regions of the brain which are rich in dendritic processes and glial cells, he is correlating morphological, electrical, and behavioral phenomena: specifically, how glial impedance shifts may modulate dendritic electrotonic current flow to form the basis of memory. He also presented data on the effects of carbon dioxide because he suspects that carbon dioxide may play some role in the ionic mechanism underlying the impedance responses.

1. Impedance Technique

Adey's technique, developed by R. T. Kado, uses microvolt signals, well below those expected to excite the tissue in which the measurement is made: current density at the electrode of 10^{-13} amp/square micron; frequency of 1 kc./sec. A coaxial type of electrode may be chronically implanted, or inserted acutely, and connected to a conventional bridge with variable resistance and capacitance for balancing. The signal is then amplified and passed to a sampling system where sampling pulses are applied (1) at the baseline crossover of the undisplaced bridge signal to give the relative resistive component and (2) at the peak of the applied signal, to yield the relative reactive component, a possible indication of membrane phenomena.

This sensitive technique provides that only signals with some relation to the original signal are detected, while processes which are random relative to the sampling window sum to zero when integrated over time. In Adey's studies, signals between 0.1 and 0.01 microvolt at the amplifier input are detected in the presence of an input noise of 3-4 microvolts, a sensitivity which permits him to see changes in impedance due to subtle physiologic activity. (See Adey, Kado, and Didio, 1962; Adey, Kado, Didio and Schindler, 1963; Porter, Adey, and Kado, 1964.)

2. EEG Technique

EEG's are followed by the same electrode used to measure impedance. Adey is using a computer to reveal

differences between EEG's, differences that are not apparent from "looking at" them. For "paradoxical" sleep, which was named from the fact that the EEG looks the same for this state as it does for the state of arousal and awakening, Adey can compute that the EEG is different. (Adey did establish that the impedances differed in the amygdala: the resistive component rising during paradoxical sleep but falling during arousal, while the cortical EEG's "looked" the same. This falling resistance may be related to Adey's universal observation that the impedance falls in response to physiological stimulation.) A computer technique used to show differences between EEG's involves the bandwidth-duration stability factor, a measure of the bandwidth of the EEG in terms of an equivalent noise. This allows one to plot an analysis of any pseudo-random process like the EEG in terms of a noise that would best specify it for that particular epoch, in terms of the bandwidth of the noise and the duration that that noise burst would last. (See Adey, 1965; Rhodes, Reite, Brown, and Adey, 1963.)

3. Impedance Shifts Caused by Environmental Stimuli

a. Hypothermia

Under the influence of hypothermia, the hippocampal resistance rises and the capacitance falls. The degree of impedance change, in general, follows the general contour of falling temperature down to 21°C. It also closely parallels carbon dioxide excretion, both in the phase of falling temperature, and in the recovery phase, when carbon dioxide continues to drop below control levels. There is no direct correlation with blood pressure in man or animals, and brain impedance has been found to be only indirectly related to blood flow in the carotid. Thus glia might form a buffer between neurons and the vascular apparatus, in which such phenomena as endogenous CO_2 production may exercise selective control on ionic exchanges that are presumably the basis of the impedance shift (see Adey, Kado, and Walter, 1965).

b. Circulatory Arrest

Upon circulatory arrest, the impedance of the hippocampus changes suddenly and enormously, an order of magnitude more than with other factors Adey discussed. The resistance rises and the capacitance falls; and if circulation is not restored the resistance continues to rise acutely. Adey said

that the electrical changes produced by spreading depression -- previously discussed by Ranck -- are of this magnitude also. Van Harreveld[10,11] had found that during asphyxia and death processes large changes in brain impedance occur which are related to changes in cerebral volume; more recently, changes in glial volume.

c. Carbon Dioxide

Hypothermia and circulatory arrest are both associated with decreased excitability of neuronal tissue; contrary effects follow modest increases in CO_2 levels in many central regions. In response to breathing seven per cent carbon dioxide in air, the animal displayed regional differences: in the amygdala, fall in the resistive component, but no change in the capacitive, giving support to Adey's view that the amygdala is a "squashy bag of ions"; in the reticular formation, moderate fall in resistance and rise in capacitance; in the hippocampus, large fall in resistance and rise in capacitance. The findings in the amygdala have been confirmed in man (Porter, Adey, and Kado, 1964).

If the animal is stimulated peripherally, as by a series of very loud noises or by squeezing the foot painfully, impedance changes occur in the hippocampus that are of equal magnitude to, and in the same direction as, the changes caused by the above CO_2 inhalation. Adey found that simultaneously with the impedance changes induced by physiological stimulation, the animal expired a slightly increased amount of CO_2 (Figure 6); yet the magnitude of the impedance changes physiologically induced paralleled those following inhalation of a much higher concentration of CO_2. This suggests, though not conclusively, that cerebral tissue is more sensitive to its endogenous CO_2 than it is to the effects of raised concentrations in inspired air.

d. Role of Glia

If these effects relate to glia, glia may serve as a barrier to prevent the escape of substances from the neuronal environment into the vascular system. The sensitivity of these impedance changes to CO_2, and the ability of carbonic anhydrase in neuroglial tissue to modify sodium and chloride fluxes into neurons via the extracellular space support the possible role of CO_2 as a regulatory substance, as suggested by Svaetechin et.al. (1963), in this respect.(28) Those

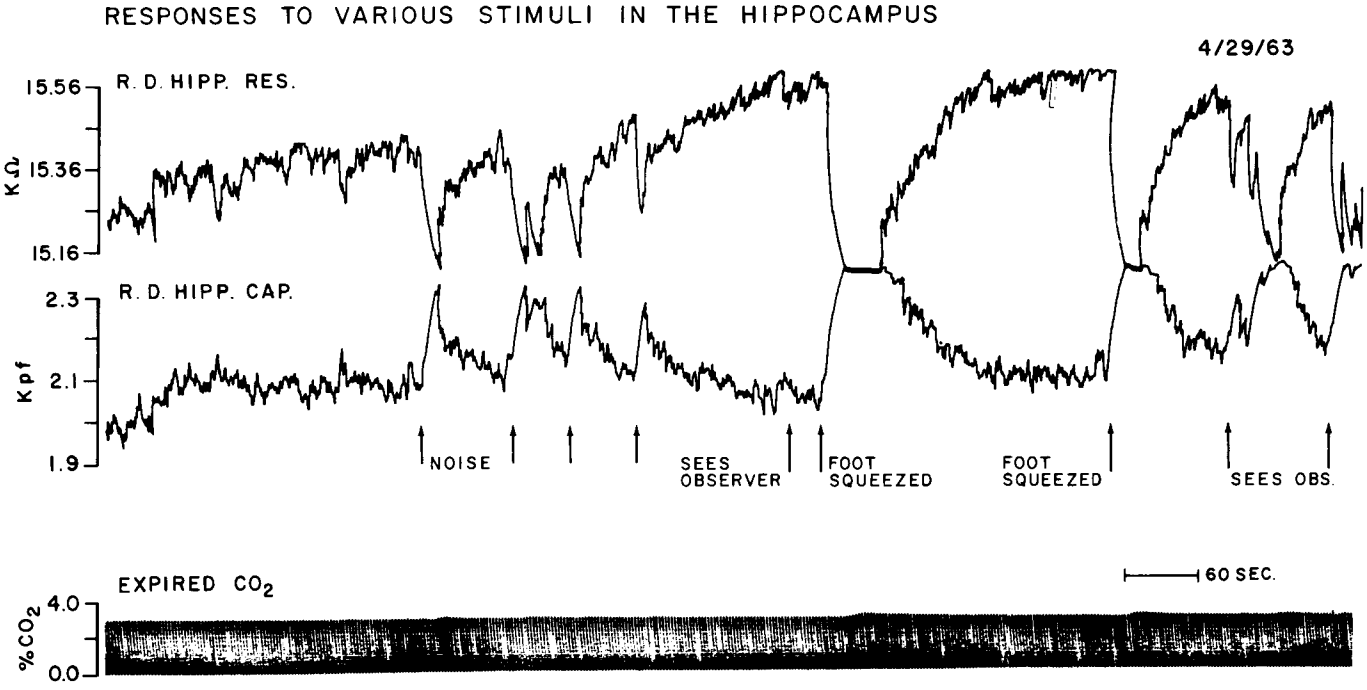


Figure 6. Simultaneous records of hippocampal resistive impedance (R.D. HIPP. RES.), expressed in Kilohms, and reactive impedance (R.D. HIPP. CAP.), expressed in Kilopicofarads. These tracings show effects of auditory, visual and somatic stimuli. A small transient increase in expired CO_2 accompanies each stimulus. From Adey, Kado, and Walter (1965), Exper. Neurol., in press.

concerned with the blood-brain barrier (Woodbury,[25] Tschirgi,[31] and others) are interested in the role of carbonic anhydrase in limiting the rate at which molecular CO_2 is converted to carbonic acid; and in the possibility that, associated with this conversion, there will be some modulation of the sodium and chloride shifts from the extracellular space.

4. Electrophysiological Correlates of Learning Behavior

A task situation has been used for some years with cats, in which the animal learns to approach food behind an opaque or transparent door, cued by light. After the animal is fully trained to approach concealed food with a light cue, the situation is reversed and the animal is retrained to approach the unlit compartment of the T-box. Over six years, Adey's group has recorded 150,000 responses from 200 cats; and some observations have been made also on monkeys and chimpanzees. This behavioral situation was selected because it turned out in practice that the animals never became habituated to the situation; the theta rhythm could be sustained through more than 5000 trials on one animal. The performance involved a complex visual discrimination, not a simple conditioned orienting reflex, as for instance in Grastyan's studies, where a simple schedule of stimulus reinforcement is used, so that habituation occurs very quickly.

a. EEG Observations

Typically and consistently, Adey sees in the EEG wave trains from the hippocampus a regularization during the animal's approach to food, in the discriminative task. In many ways rhythms from this and other parts of the brain of the cat are as specific as thumbprints, and highly indicative of the region of the brain from which they come. A marked regularization occurs during the discrimination in the hippocampus and in the entorhinal cortex; a more variable rhythm, in the subthalamus and midbrain reticular formation; and a less sustained rhythm, in the visual cortex. These processes center around a frequency of 6 cycles per second in most cats. (A region-specific rhythm in the amygdala at 35-40 cycles per second is related to general state of alertness, not discrimination.)

A variety of computing techniques have been used to better specify the wave processes. Adey presented the simplest

of these: a daily computer average of the EEG processes in the hippocampus occurring during repeated trials by the animal. At 75 per cent correct performance level, an average was essentially irregular; but three weeks later, at about the same performance level, the animal's averaged EEG became highly regular. This regularity was thereafter sustained as long as the animal retained his discriminating performance. Then, immediately following the cue reversal, at about 10 per cent correct performance on the first post-reversal day, the averaged EEG was excessively regular, as if he were trying to force some signal system to perform in a way that it had performed previously. Thereafter the regularity declined as performance went through the chance range, and finally another regular average appeared as 90 per cent correct performance was attained. Some similar regularities also occur in the midbrain.

b. Related Impedance Observations (see Fig. 7)

In the hippocampus the averaged resistive component of the complex impedance decreased characteristically as the EEG regularized during approach (multiple implants, recording impedance on one and EEG from adjacent leads). At the chance level of performance where the EEG does not appear very regular, the impedance shows no response during the task performance. At 80 per cent correct response, the impedance falls slightly at the onset of the task performance and then rises for 1 to 2 seconds. At 100 per cent performance, the impedance decreases profoundly during approach and then rises before returning to the baseline value -- in its full configuration, an S-shaped curve. This persists in the hippocampus as long as the animal retains the learned performance. Then, after cue reversal and initiation of retraining on the first day when the hippocampal EEG is hypersynchronized, the evoked impedance response is even larger. In subsequent retraining, the impedance response disintegrates, to reappear as performance capability rises above chance levels, to the point of such subtleties as the hippocampal impedance falling earlier in time to anticipate the door opening slightly. Some less clear deflections occur in the midbrain, but no correlations with task performance have been detected in the amygdala.

5. Brain Impedance Changes after Lesions: Baseline Shifts due to Glia

a. Retrograde Lesions: Lateral Geniculate Body

Reactive and resistive impedances were followed from

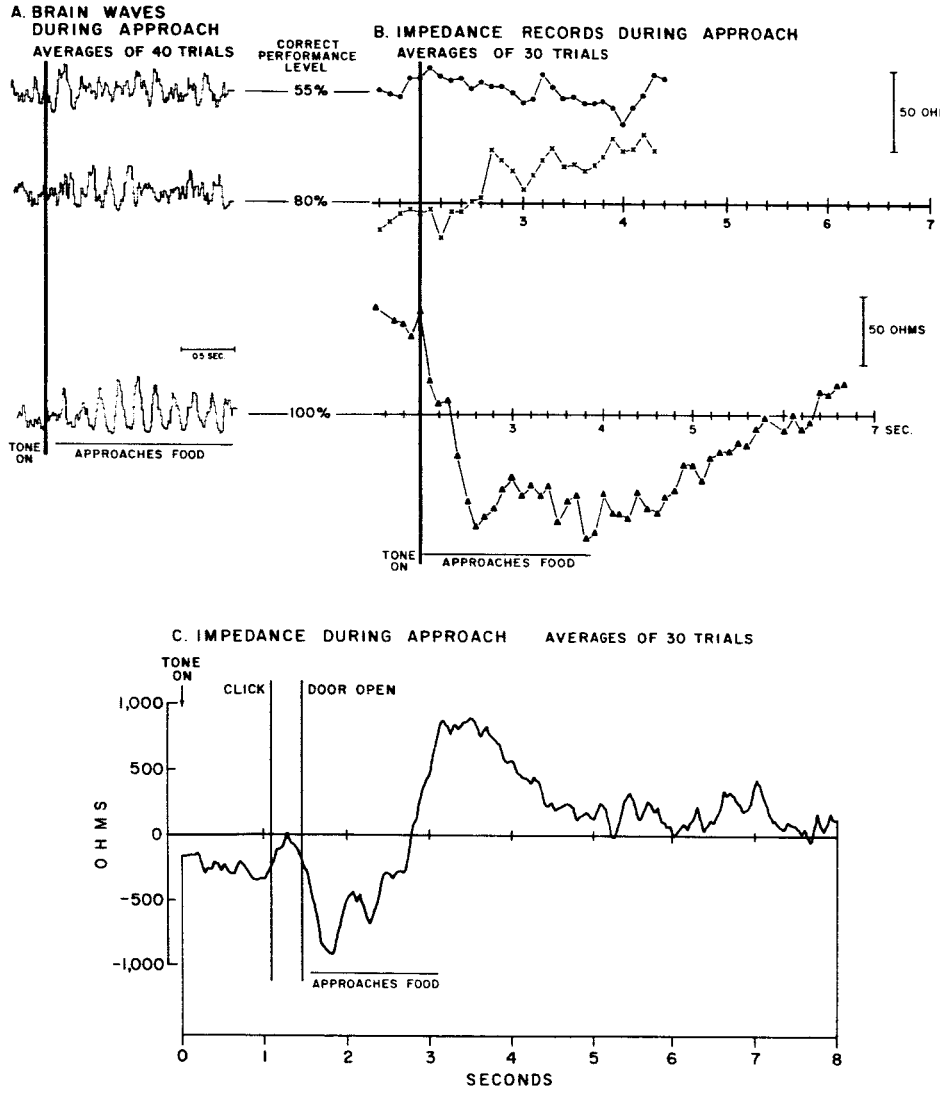


Figure 7. Characteristic decrease in hippocampal impedance with regularization in EEG during discriminative performance (from Adey and Kado, Scientific American, in press).

four coaxial-type electrodes placed in anterior and posterior parts of both lateral geniculate bodies. Two months after implantation, when the baseline impedance became stable, the right visual cortex was resected. Beginning one week later, and for the ensuing three weeks, the impedance in the posterior of the right lateral geniculate shifted substantially from 12 to 28 kilohms in the resistive component and then returned to the baseline value prior to the lesion. There was very little change in the recordings from the opposite side except for one unexplainable transient shift ten days after the lesion. Adey suspects that much of the changes relate to the alteration in the characteristics of the glia in the course of the degenerative process. This would agree with recent papers in Exp. Neurol.[20] and J. Anat. London[23] on the effects of retrograde and transneuronal degeneration in the lateral geniculate, indicating heavy participation of glia in this response.

b. Irradiation: Hippocampus

After bilateral radiation (2500 r, single dose) of the hippocampus of the cat, progressively increasing perturbations occurred in the baseline of the impedance, with peaks approximating twice the baseline values (Schoenbrum, Campeau, and Adey, 1963). This is associated with an alteration in cell content and the dropping out of neurons with alterations in glial behavior, and has nothing to do with vascularity since necrosis with blood vessel involvement occurred later.

6. Human Brain Impedance

Electrodes were implanted in six patients for diagnostic and therapeutic reasons (four temporal lobe epileptics and two Parkinsonian patients) in temporal lobe structures -- amygdala, hippocampus -- and in the thalamus. Implantation in the midbrain reticular formation, as done with animals, was not considered justifiable in man.

Human amygdala capacitance was unresponsive to carbon dioxide, as was the case for animals, but amygdaloid resistance was lowered in both animals and man with carbon dioxide.

There is a reversal of the effect of hyperventilation during the post-operative period: three days after implantation, hyperventilation causes a rise in resistance and a fall in capacitance, whereas twelve days after operation in the same patient the resistance fell and the capacitance rose.

This probably relates to the presence of edema fluid and cellular disruption in the early post-operative phase.

Occlusion of both carotids to the point of clouding of the patient's consciousness caused very minor perturbations in resistance and no change in the capacitance. This, along with observations of postural effects, indicate that Adey's measure of brain impedance does not relate directly to blood flow.

In the course from wakefulness to sleep there are a gradual rise in resistance and a fall in capacitance, with transient sharp falls in impedance during dream phases.

In patients with abnormal hippocampal tissue on one side, it was consistently found that the resistance is lower and the capacitance higher in the abnormal tissue in the early days after the operation, though the values tend to come together in 10 to 14 days.

The impedance of normal tissue takes longer to recover from hyperventilation than does abnormal -- 20 minutes versus 6-8 minutes.

7. Glial Role in Memory

Adey explained that regions of the nervous system that are implicated in the ready storage of information are characterized morphologically by having layers of profoundly-overlapping dendritic trees (from neuron somata in other layers) with glial cells interspersed among the dendrites. D. A. Sholl[27] was one of the first to draw attention to the fact that dendritic overlap in cortical fields was one of the most characteristic aspects of the organization of the cortex. It is a phyletic difference in that the higher the animal, the greater the volume of dendrites in proportion to the volume of neuron somata. The proximity of at least some dendrodentritic associations, according to van der Loos,[21] is of the order of Angstroms. This phenomenon of dendritic overlap is particularly true of the hippocampus, and also occurs in the thalamus, and in some degree in the reticular formation. The spinal cord, which in Adey's view is incapable of storing information in the sense of a recallable memory, has no such characteristics.

Associated with this type of structure is a characteristic electrical pattern of regular wave processes, which may

be associated with synaptic potentials or so-called dendritic potentials which sweep in an electrotonic fashion from the tips of the dendrites toward the cell bodies, but which in any case have extraneuronal components. Adey would infer that the impedance changes may relate to a modulation of the electrotonic phenomena in dendrites by glia. Changes in the glial impedance load might thereby alter the frequencies of the EEG patterns. This change in impedance load could have both spatial and temporal non-linearities.

Extrapolating from other data not presented at the Work Session, Adey believes that the wave patterns are associated with the initial deposition of information on a permanent basis in brain tissue, and that the recall of the information is associated with the reappearance of similar, but not necessarily identical, wave process on a "best-fit" basis. Although he has not yet analyzed the more complex EEG patterns completely, he can say that there is a definite difference in pattern in the learning experiment between the EEG's for correct and incorrect responses (Adey and Walter, 1963; Walter and Adey, 1963).

The hypothesis suggests that dendritic current flow may be the physical basis for subsequent chemical events in which the membranes of glial cells and neurons are mutually altered on a permanent basis. The threshold of a particular group of cells would then be lowest in the presence of a pattern of waves resembling the one that produced the original change: a frequency-modulated ion flux that evokes the same thing that laid it down.

Adey sees the hippocampus as a sort of overviewer of the function of information recall, not the site of memory.*

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* Compare this to Robert B. Livingston's metaphor of the hippocampus as the "city editor" that issues "Now print!" orders with regard to particular memories. [See Nauta, W.H. Some Brain Structures and Functions Related to Memory: NRP Work Session Report, NRP Bulletin 2(5):19 (Sept-Oct 1964).]

IV. MYELINATION, DEMYELINATION AND MEMORY

A. Tissue Culture Experiments

Bornstein presented work with modified organ cultures of rat cerebellum to study the processes of myelination and demyelination to better elucidate the mechanisms involved in the demyelinating diseases. He observes the system by ordinary bright field illumination. His tissue cultures maintain several realistic in vivo characteristics: EM studies (Leonard Ross) of selected, myelinating cultures reveal glial membranes wrapping around axons; though he has not seen morphological differences between astrocytes and oligodendrocytes. Synapses and nodes were present.

1. Experimental Allergic Encephalomyelitis

Bornstein described a series of experiments in which sister cultures from littermates are randomly exposed to various sera. He emphasized the delicacy of the cultures and the need for controls, yet cultures were unaffected by a rabbit anti-rat kidney serum which was highly nephrotoxic to rats. A culture exposed to rabbit "EAE" serum, however, changed in a number of ways which were found to be characteristic (Fig. 8). Effects on the culture included swelling and displacement of neuroglia, fusiform swellings of myelin sheaths, and breakdown of myelin with formation of fat droplets. Of the sera tested in this series, only serum from animals who had, or were about to have, experimental allergic encephalomyelitis produced these changes. Antisera from mouse, rat, guinea pig, and rabbit have been used on mouse and rat cultures.

Bornstein believes that antibody is involved since the 7S part of the globulin fraction produced the effect when applied in the presence of complement, and fluorescence studies showed duck anti-rabbit-globulin globulin adhering to the glial cell membranes and to the myelin. Complement is also considered necessary since the usual temperature and pH changes inactivated the responsible factor, and addition of normal serum to inactivated serum renewed the demyelinating effect.

2. Multiple Sclerosis (MS)

Similar effects on rat cerebellum culture, including fusiform swelling of sheaths, swelling of glia, and breakdown

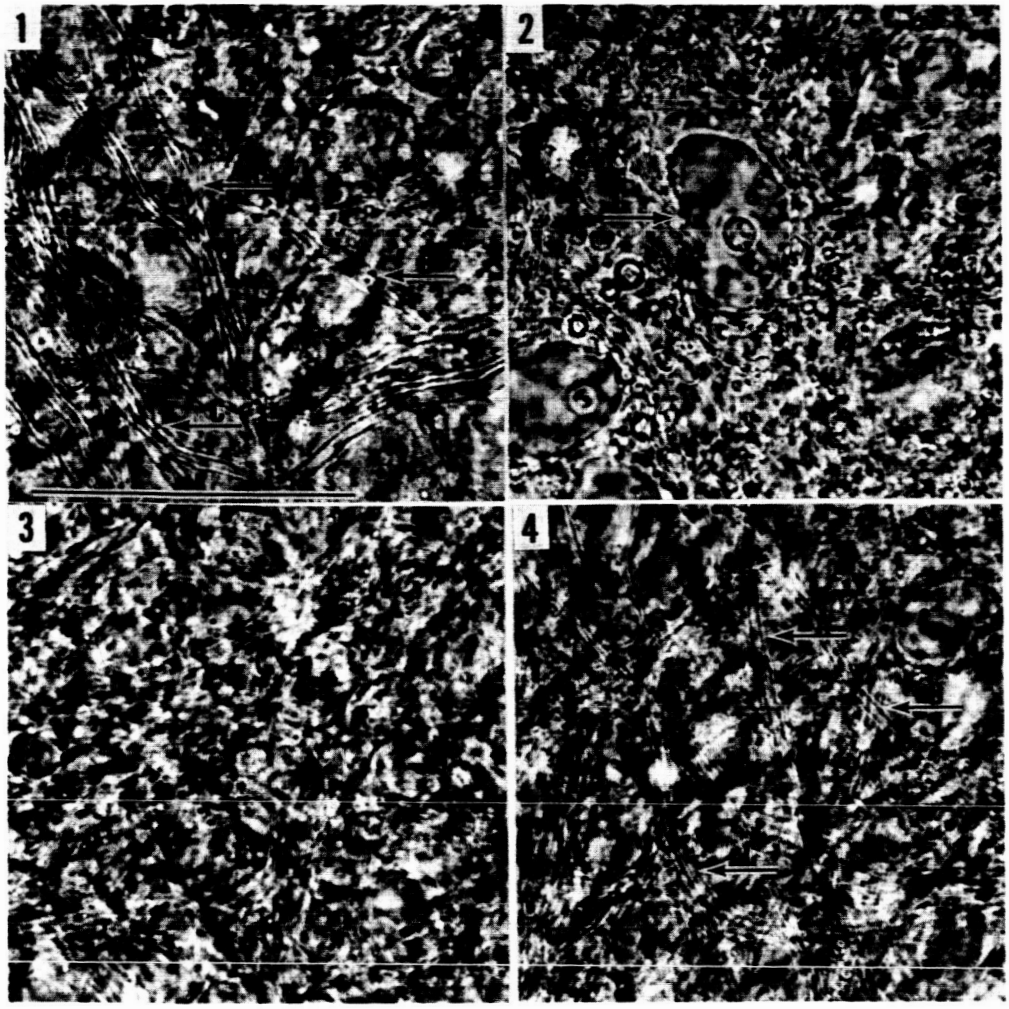
Figure 8. All figures represent photographs of a living unstained culture of rat cerebellum as seen by ordinary bright-field illumination and photographed at a magnification of 600 diameters. The scale at the lower border of (1) represents 50 microns.

(1) Control period of culture on the 16th day *in vitro* (DIV). Arrows marked "A," "B" and "C" designate axons which will remyelinate (*cf*. (4)).

(2) Identical area of the same culture, but after 4 1/2 hours after exposure to a 20% concentration of serum from a rabbit suffering from experimental allergic encephalomyelitis. All myelin sheaths have disappeared leaving only a few fragments undergoing further lipolysis. Arrows "G" designate swollen, dead neuroglia.

(3) The same area photographed 24 hours after a 24 hour exposure to the EAE serum. The culture has been returned to its normal nutrient medium. Note the disappearance of the dead neuroglia, the scattered fat droplets and the difficulty with which denuded axons may be visualized against the neuroglial background.

(4) Twelve days after demyelination (28 DIV) and return to normal *in vitro* conditions. Remyelinated axons, as shown by arrows, are slightly displaced by the previous neuroglial swellings and rearrangements.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of myelin, were caused by incubation with sera from a majority of multiple sclerosis patients. 220 sera have been tested. The effect of the serum even correlated with the stage of clinical activity of the disease. Of sera from patients in stages of exacerbation, about two-thirds demyelinated the tissue culture, whereas of those with questionable clinical activity, only one-third showed an effect. No sera from patients in remission had any effect. Twenty-six normal subjects tested negative, although two false positives were noted; and sera from patients with destructive lesions of the central nervous system which were not essentially demyelinating did not show the effect.

Asked whether he would conclude on the basis of his observations that experimental allergic encephalomyelitis and multiple sclerosis are related conditions, Bornstein stated that the studies support this hypothesis, but he could not yet say whether the factors in MS sera might not be the result rather than the cause of the disease.

3. Remyelination

Bornstein found that, after the serum was washed off the culture, the fibers were able to remyelinate, and that this alternation of myelination and demyelination could be repeated in the same culture as many as three times. The remyelination, however, was almost never complete; and the myelin sheath tended to be segmented and not as thick as in normal sister cultures.

Dr. Levine raised a question that could not be answered: how this could be an antibody-antigen reaction and be reversible. It was suggested that something may happen to concentrate complement at the cell membrane or that the surface is sensitized non-specifically, as is known for red blood cells. Dr. Galambos, however, said that there is definite evidence (Kies[17]) that a basic protein derived from the myelin itself serves as a powerful antigen, though it may not be the only one.

4. Neocortex Organ Culture

Bornstein was urged by the Work Session attendees to describe a new culture of neocortex from neonatal mice since it would appear to be an excellent model system for the study of development as well as of environmental effects. The

explant is 2 mm. cortex surface x 1 mm. deep x 1/2 mm. thick and has maintained its gross architecture for three months. As would occur in the live animal, the packing density of the neurons decreases with time; myelination occurs in 2-3 weeks in the area in which white matter would be expected to develop; neurons grow in size; synapses form. Crain* is following development of electrical activity: from no activity at first, then at 3-4 days, tenths-of-millisecond spike responses to stimulus; at 5-6 days, an added prolonged discharge of a few hundred milliseconds; about a week later, afterdischarges. Furthermore the recordings from the depth of the explant are positive in charge, and the surface negative, as in the animal. Various neuropharmacological studies have begun.

B. Phenylketonuria (PKU)

Akert briefly described investigations on phenylketonuria begun two years ago, though not followed through. As far as the experiments were carried, they indicated that PKU is a glial disease and sometimes also a myelination disorder.

The work was started because of the anomaly of severity of psychological symptoms yet absence of noticeable neuropathological symptoms. With Dr. Waisman of the Wisconsin Psychology Department, Akert produced a chemically similar condition in rats by feeding them high doses of L-phenylalanine. Levels of the compound dropped in the liver, rose in the plasma, and were excreted in the urine. As a result intelligent behavior was strikingly disturbed -- timing, orienting, and other behavior; but the light microscope revealed no striking changes in the brain tissue.

In the early stages of this disease, electron microscopy did reveal significant and consistent changes, always in the glial cells, both in cerebral and in cerebellar cortex. Astrocyte mitochondria were considerably enlarged; the positions of the cristae were irregular; and granules were present in the matrix -- the more progressed the disease, the more granulation. But abnormalities were never found in neurons.

Akert also found in phenylalanine-treated animals

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* See the forthcoming report of the NRP Work Session on the Synapse by its Chairman, J. David Robertson.

characteristic signs of retarded myelination. Friede added that he had examined three human brains from PKU patients who had been considered psychologically to be idiots, and only one of the three had a unique pattern of symmetrical incomplete myelination in both parietal lobes that looked like shadow plaques by light microscope.

Bornstein reported that he had cultured cerebellum from a recessive sex-linked leukodystrophy in a litter of nine mice and found that two of the nine had less myelin than the others.

Galambos commented that more work is needed on the relation between myelination and glial abnormalities.

C. Multiple Sclerosis: Psychological Measurements

Williams found that although there is a large clinical literature on MS there are few systematic quantitative studies. There are several important problems in the examination of the MS patient which make the interpretation of psychological test results difficult. First, test results usually can not be correlated with pathological findings since the illness does not significantly shorten the patient's life span; second, it is rarely possible to obtain pre-morbid measures of performance; third, the widely disseminated lesions of MS often cause specific motor impairments which interfere with performance and make the evaluation of performance-based tests difficult.

Most of the 15 quantitative studies found by Williams relied on I. Q. and projective tests for the evaluation of psychological status. Diers and Brown (1950),[7] using the Wechsler-Bellevue test, found that MS patients consistently made lowest scores on Digit-Span, a test which requires the subject to memorize and immediately recall sequences of digits. Harrower and Kraus (1951),[12] with the Wechsler-Bellevue and Rorschach tests, found a general inverse correlation between performance and severity of illness. They also found consistent impairment on the Digit-Span test. On the other hand, their patients regularly showed highest scores on the Information subtest. This test taps information which is usually acquired in school. In 1952, Baldwin,[2] using the Hunt-Minnesota test, showed that MS patients performed worse than matched controls on problems requiring the learning and the immediate recall of words and geometric forms.

All of the above studies were cross-sectional in that they relied on normal control groups for estimating deficit in multiple sclerosis. The only investigator able to obtain direct measures of impairment was Cantor (1951).[6] He studied a group of veterans who had been given the army AGTC test prior to the onset of MS. These patients showed a substantial decline in performance when examined about two years after the onset of MS, and the amount of impairment correlated with judgements of severity of illness. Other investigators (e.g. Parsons et al, 1957) have shown that MS causes deficits in a variety of psychological functions, including abstract reasoning. The most consistent defect, however, appears on tests involving recent memory.

Williams saw some similarities in these MS results to his own studies of sleep loss. A sleep deprived subject can retain and retrieve information learned prior to sleep deprivation. As he becomes drowsy, however, he has increasing difficulty with the acquisition and retrieval of new information.

1. Discussion

Galambos mentioned the problem that most of the psychological tests used in these studies were really intended to predict school grades. They were not designed for the specific purpose of measuring impaired performance. Williams agreed, and pointed out that there are very few laboratories working on the development of specific measures of impaired performance. He suggested that experimental tests now used in studies of stress, fatigue, sleep loss, and drug states might be usefully applied to the examination of clinical patients.

Asked to comment on behavioral tests of memory in man, Williams suggested that the development of such measures depend on a psychological model for memory. For his own work on performance, he has found information-processing models to be usefuly. Key psychological functions implied by these models include sensing, selecting, encoding, storing, and retrieving information. A general problem for the investigator of clinical states is to determine which of the several data-processing functions is impaired. Systematic variation of information load, speed load, retrieval or response requirements may permit more precise inferences about the nature of psychological deficit.

V. RECOMMENDED FURTHER READING

/EDITOR'S NOTE: The bibliography that follows was generated by the participants in the Work Session. Section A lists publications written by themselves and by others that they recommended in advance of the meeting; section B lists citations made during the meeting. This bibliography has been checked and updated by participants--as has this entire report--as recently as February, 1965, and constitutes a selection chosen by experts as key papers in the field, intended as an aid at least for the scientific newcomer to glial studies, and possibly even for the more knowledgeable professional.

As an additional aid to the investigator, the NRP intends soon to publish a working list of about 1000 titles of papers relevant to glial cells, published or abstracted in the 1960's. It is to be a supplement to this issue, mailed under separate cover. -- CML./

A. Listed by Individual Participant

W. ROSS ADEY

1. Own Relevant Publications

Adey, W. R., Kado, R. T., and Walker, D. D. (1965): Impedance characteristics of cortical and subcortical structures: evaluation of regional specificity in hypercapnea and hypothermia. Exp. Neurol. (in press, will appear Feb., 1965)

Adey, W. R. (1965): Computer analysis in neurophysiology. In: Computers in Biomedical Research. Vol. I. Stacy, R., and Waxman, B., eds. New York: Academic Press. pp. 223-263.

Porter, R., Adey, W. R., and Kado, R. T. (1964): Measurement of electrical impedance in the human brain. Neurology 14:1002.

Porter, R., Adey, W. R., and Brown, T. S. (1964): Effects of small hippocampal lesions on locally recorded potentials and on behavior performance in the cat. Exp. Neurol. 10: 216.

Schoenbrum, R., Campeau, E., and Adey, W. R. (1963): EEG and behavioral effects from X-irradiation in the hippocampal system. In: Effects of Ionizing Radiation on CNS. (Second Symposium, Los Angeles) Snider, R., and Haley, T., eds. (in press)

Walter, D. O. (1963): Spectral analysis for electroencephalograms: mathematical determination of neurophysiological relationships from records of limited duration. Exp. Neurol. 8:155-181.

Adey, W. R., and Walter, D. O. (1963): Application of phase detection and averaging techniques in computer analysis of EEG records in the cat. Exp. Neurol. 7:186.

Adey, W. R., Kado, R. T., Didio, J., and Schindler, W. J. (1963): Impedance changes in cerebral tissue accompanying a learned discriminative performance in the cat. Exp. Neurol. 7:259.

Rhodes, J. M., Rite, M. R., Brown, D., and Adey, W. R. (1963): Cortico-subcortical relationships of the chimpanzee. In: Proceedings of the CNRS Symposium on Sleep Mechanisms. (Lyons, France) (in press)

Adey, W. R., Walter, D. O., and Lindsley, D. F. (1962): Subthalamic lesions. A. M. A. Arch. Neurol. 6:34.

Adey, W. R., Walter, D. O., and Lindsley, D. F. (1962): Effects of subthalamic lesions on learned behavior and correlated hippocampal and subcortical slow-wave activity. A. M. A. Arch. Neurol. 6:194.

Adey, W. R. (1962): EEG studies of hippocampal system in the learning process. (Int. Physiologie de l'hippocampe, 1962) Colloques Internat. Ctr. Natl. Reh. Sci. 107:203.

Adey, W. R., Kado, R. T., and Didio, J. (1962): Impedance measurements in brain tissue of animals using microvolt signals. Exp. Neurol. 5:47.

2. Other Suggested Background References

Aladjalova, N. A. (1964): Slow electrical processes in the brain. In: Progress in Brain Research. Vol. VII. Amsterdam: Elsevier. (in press)

Aladjalova, N. A. (1955): Metod odnovremennogo izmerenia omicheskoe i emkostnoi sostavlavushemikh impedantsa vo vremia bistrogo nestatsionarnogo protsessa. Tr. Ir-ta. Biophiziki 1:298-305.
"Its contents are closely related to impedance and slow potential measurements." (W. R. A.)

Cole, K. S. (1940): Permeability and impermeability of cell membranes for ions. Cold Spring Harbor Symposia Quant. Biol. 4:110-122.

Harreveld, A. van, Hooper, N. K., and Cusick, J. T. (1961): Brain electrolytes and cortical impedance. Amer. J. Phyiol. 201:139-143.

Hydén, H., and Pigou, A. (1960): A cytophysiological study of the functional relationship between oligodendroglial cells of Deiters' nucleus. J. Neurochem. 6:57-72.

Rall, W. (1962): Electrophysiology of a dendritic neuron model. Biophysical J. 2:145-167.

KONRAD AKERT

1. Own Relevant Publications

Akert, K., Potter, H. D., and Anderson, J. W. (1961): The subfornical organ in mammals. V. Comparative and topographical anatomy. J. Comp. Neurol. 116:1-14.

Akert, K., Hable, K., Wang, H. L., and Waisman, H. A. (1961): Ultrastructural cerebral changes in experimental phenylketonuria. In: Reports of the VIIth International Congress of Neurology. pp. 29-32.

2. Other Suggested Background References

Waisman, H. A., Hable, K., Wang, H. L., and Akert, K. (1964): Some ultrastructural changes in the brain of phenylketonuric rats and monkeys. In: Progress in Brain Research. Vol. IX. Amsterdam: Elsevier. pp. 207-212.

Spoerri, O. (1963): Ueber die Gefässversorgung des Subfornikalorgans der Ratte. Acta Anat. 54:333-348.
"This is an MD thesis on vascular patterns of a neuroglial-vascular structure." (K. A.)

Landolt, A. (1965): Elektronenmikroskopische Untersuchungen am Rindengrau der Corpora pedunculata von Waldameisen (Formica lugubris Zett.) mit besonderer Berücksichtigung der Neuron-Glia-Beziehung. Z. Zellforsch. (in press)
"Describes glial morphology in ant brain cortex." (K. A.)

JOSEPH ALTMAN

1. Own Relevant Publications

Altman, J., and Das, G. D. (1964): Autoradiographic and histological investigation of changes in the visual system of rats after unilateral enucleation. Anat. Rec. 148:535.

Altman, J., and Das, G. D. (1964): Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. Nature 204:1161.

Altman, J. (1963): Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anat. Rec. 145:573.

Altman, J., and Altman, E. (1962): Increased utilization of an amino acid and cellular proliferation demonstrated autoradiographically in the optic pathways of pigeons. Exp. Neurol. 6:142.

Altman, J. (1962): Autoradiographic study of degenerative and regenerative proliferation of neuroglia cells with tritiated thymidine. Exp. Neurol. 5:302.

Altman, J. (1962): Are new neurons formed in the brains of adult mammals? Science 135:1127.

2. Other Suggested Background References

Cammermeyer, J. (1960): Reappraisal of the perivascular distribution of oligodendrocytes. Amer. J. Anat. 106:197-231.
"Excellent bibliography." (J. A.)

Glees, P. (1955): Neuroglia, Morphology and Function. Springfield, Ill.: C. C. Thomas.
"Good summary of relevant data and interpretations up to date of publication." (J. A.)

Smart, I. (1961): The subependymal layer of the mouse brain and its cell production as shown by radioautography after thymidine-H^3 injection. J. Comp. Neurol. 116:325-347. "Stresses the importance of the subependymal layer of the lateral ventricle as a site of ongoing cell multiplication in the young and adult rodent brain." (J. A.)

Smart, I., and Leblond, C. P. (1961): Evidence for division and transformations of neuroglia cells in the mouse brain, as derived from radioautography after injection of thymidine-H^3. J. Comp. Neurol. 116:349-367.

Windle, W. F., ed. (1958): Biology of Neuroglia. Springfield, Ill.: C. C. Thomas.
"A more up-to-date source of materials on glia." (J. A.)

RUSSELL J. BARRNETT

1. Own Relevant Publications

Marchesi, V. T., Sears, M. V., and Barrnett, R. J. (1964): Electron microscopic studies of nucleoside phosphatase activity in blood vessels and glia of the retina. Invest. Ophthal. 3:1.

Torack, R. M., and Barrnett, R. J. (1964): The fine structural localization of nucleoside phosphatase activity in the blood-brain barrier. J. Neuropath. Neurol. 23:46.

Barrnett, R. J. (1963): Localization of enzymatic activity at a fine structural level. Proc. Roy. Micro. Soc. 82:1.

Barrnett, R. J. (1963): Fine structural basis of enzymatic activity in neurones. (Symposium - Fine Structure and Function of the Neurone) Trans. Amer. Neurol. Assoc. p. 123.

Marchesi, V. T., and Barrnett, R. J. (1963): The demonstration of enzymatic activity in pinocytic vesicles of blood capillaries with the electron microscope. J. Cell Biol. 17:547.

Marchesi, V. T., and Barrnett, R. J. (1963): The localization of nucleoside phosphatase activity in different types of small blood vessels. J. Ultrastruct. Res. 10:103.

Torack, R. M., and Barrnett, R. J. (1963): Nucleoside phosphatase activity in neurons and glia investigated by electron microscopy. J. Histochem. Cytochem. 11:763.

Torack, R. M., and Barrnett, R. J. (1962): Fine structural localization of cholinesterase activity in the rat brain stem. Exp. Neurol. 6:224.

MURRAY B. BORNSTEIN

1. Own Relevant Publications

Appel, S. H., and Bornstein, M. B. (1964): The application of tissue culture to the study of experimental allergic encephalomyelitis. II. Serum factors responsible for demyelination. J. Exp. Med. 119:303.

Bornstein, M. B. (1963): A tissue culture approach to demyelinative disorders. N. C. I. Monograph No. 11:197.

Bornstein, M. B., and Appel, S. H. (1961): The application of tissue culture to the study of experimental "allergic" encephalomyelitis. I. Patterns of demyelination. J. Neuropath. Exp. Neurol. 20:141.

Bornstein, M. B., and Murray, M. R. (1958): Serial observations on patterns of growth, myelin formation, maintenance and degeneration in cultures of newborn rat and kitten cerebellum. J. Biophys. Biochem. Cytol. 4:499.

Bornstein, M. B. (1946): Presence and action of acetylcholine in experimental brain trauma. J. Neurophysiol. 9:349.

2. Other Suggested Background References

Peterson, E. R. and Murray, M. R. (1955): Myelin sheath formation in cultures of avian spinal ganglia. Amer. J. Anat. 96:319.
"First description *in vitro* myelination." (M. B. B.)

Hild, W. (1957): Myelogenesis in cultures of mammalian central nervous tissue. Zeitschr. f. Zellforsch. 46:71.
"First description of *in vitro* myelination of mammalian CNS." (M. B. B.)

Harrison, R. G. (1907): Observations on the living developing nerve fiber. Anat. Rec. 1:116; Proc. Soc. Exp. Biol. Med. 4:140.
"First tissue culture of nerve." (M. B. B.)

Kies, M. W., and Alvord, E. C. (1959): "Allergic" Encephalomyelitis. Springfield, Ill.: C. C. Thomas.
"General background of 'allergic' encephalomyelitis and its possible relationship to human disorders." (M. B. B.)

REINHARD L. FRIEDE

1. Own Relevant Publications

Friede, R. L., and Knoller, M. (1964): Proximo-distal increase of enzymic activity in the dorsal spinal tracts. J. Neurochem. 11:679.

Friede, R. L., and Fleming, L. M. (1963): A mapping of the distribution of lactic dehydrogenase in the brain of the rhesus monkey. Amer. J. Anat. 113:215.

Friede, R. L. (1962): The cytochemistry of normal and reactive astrocytes. J. Neuropath. Exp. Neurol. 21:471.

Friede, R. L., and Fleming, L. M. (1962): A mapping of oxidative enzymes in the human brain. J. Neurochem. 9:179.

Friede, R. L., and Houten, W. H. van (1962): Neuronal extension and glial supply (functional significance of glia). Proc. Nat. Acad. Sci. U. S. A. 48:817.

Friede, R. L., and Magee, K. R. (1962): Alzheimer's disease. Neurology 12:213.

Friede, R. L. (1961): Enzyme histochemical studies in multiple sclerosis. A. M. A. Arch. Neurol. 5:103.

Friede, R. L. (1961): A histochemical study of DPN-diaphorase in human white matter; with some notes on myelination. J. Neurochem. 8:17.

Friede, R. L. (1961): Enzymes d'oxydation du système nerveux central. World Neurol. 2:1094.

ROBERT GALAMBOS

1. Own Relevant Publications

Galambos, R. (1964): Research in demyelinating diseases: a report of a recent conference. NRP Bulletin 2(2):97-104.

Galambos, R. (1962): Glia, neurons, and information storage. In: Macromolecular Specificity and Biological Memory. Schmitt, F. O., ed. Cambridge, Mass.: MIT Press. Chap. 13, pp. 52-54.

Galambos, R. (1961): A glial-neural theory of brain function. Proc. Nat. Acad. Sci. U. S. A. 47(1):129.

Galambos, R., and Morgan, C. T. (1960): The neural basis of learning. In: Handbook of Physiology - Neurophysiology, III. Washington, D. C.: American Physiological Society. pp. 1471-1500.

2. Other Suggested Background References

Geiger, R. S. (1963): The behavior of adult mammalian brain cells in culture. Int. Rev. Neurobiol. 5:1-52. "Numerous references to glia and neurons in culture to complement Dr. Pomerat's contribution." (R. G.)

CHARLES M. POMERAT

1. Own Relevant Publications

Pomerat, C. M. (1961): Cinematography in the service of neuropathology. In: Proceedings, Fourth International Congress of Neuropathology. (Munich, 4-8 Sept. 1961) Vol. II. Jacob, H., ed. Stuttgart: Georg Thieme Verlag, 1962. pp. 207-216.

Pomerat, C. M. (1961): Cinematography, indispensible tool for cytology. Int. Rev. Cytol. 11:307-334.

Pomerat, C. M. (1958): Functional concepts based on tissue culture studies of neuroglia. In: Biology of Neuroglia. Windle, W. F., ed. Springfield, Ill.: C. C. Thomas. Chap. 10, pp. 162-175.

2. Other Suggested Background References

Hild, W. (1959): Myelin formation in cultures of mammalian central nervous system. In: The Biology of Myelin. Korey, S. R., ed. New York: Hoeber-Harper. pp. 188-200.

JAMES B. RANCK, JR.

1. Own Relevant Publications

Ranck, J. B., Jr. (1964): Synaptic "learning" due to electro-osmosis: a theory. Science 144:187.

Ranck, J. B., Jr. (1964): Specific impedance of cerebral cortex during spreading depression, and an analysis of neuronal, neuroglial, and interstitial contributions. Exp. Neurol. 9:1.

Ranck, J. B., Jr. (1963): Specific impedance of rabbit cerebral cortex. Exp. Neurol. 7:153.

Ranck, J. B., Jr. (1963): Analysis of specific impedance of rabbit cerebral cortex. Exp. Neurol. 7:153.

Koch, A., Ranck, J. B., Jr., and Newman, B. L. (1962): Ionic content of the neuroglia. Exp. Neurol. 6:186.

2. Other Suggested Background References

Katzman, R. (1961): Electrolyte distribution in mammalian central nervous system. Are glia high sodium cells? Neurology 11:27-36.
"Gliomas have much higher Na - K rations than whole brain, indicating that glia are high sodium cells." (J. B. R.)

Adey, W. R., Kado, R. T., and Didio, J. (1962): Impedance measurements in brain tissue of animals using microvolt signals. Exp. Neurol. 5:47-66.
"Impedance of hippocampus changes with sleep and anesthesia." (J. B. R.)

Hild, W., and Tasaki, I. (1962): Morphological and physiological properties of neurons and glia cells in tissue culture. J. Neurophysiol. 25:277-304.
"Glial cells show graded additive depolarization in an external field, and repolarize over seconds. Their membrane resistance is 5 ohm cm^2." (J. B. R.)

Svaetichin, G., Laufer, M., Metarai, G., Fatehchand, R., Vallecalle, E., and Villegas, J. (1961): Glial control of neuronal networks and receptors. In: The Visual System; Neurophysiology and Psychophysics. Jung, R., and Kornhuber, eds. Berlin: Springer-Verlag.
"Glial cells are involved in the slow electrical activity of the retina." (J. B. R.)

Villegas, R., Villegas, L., Gimenez, M., and Villegas, G. M. (1963): Schwann cell and axon electrical potential differences. Squid nerve structure and excitable membrane location. J. Gen. Physiol. 46:1047-1064.
"The Schwann cell of squid axons is electrically isolated from the axon and has no active part in the action potential." (J. B. R.)

Kuffler, S. W., and Potter, D. (1964): Glia in the leech central nervous system, physiological properties and neuron - glia relationships. J. Neurophysiol. 27:290.
"There are low resistance contacts between adjacent glial cells in the leech, but no participation in neuronal activity was observed." (J. B. R.)

CLARK T. RANDT

1. Own Relevant Publications

Collins, W. F., and Randt, C. T. (1961): Fibre size and organization of afferent pathways. Arch. Neurol. 5:100.

Collins, W. F., and Randt, C. T. (1960): Midbrain evoked responses relating to peripheral unmyelinated or C fibres in cat. J. Neurophysiol. 23:47.

2. Other Suggested Background References

Langworthy, O. R. (1933): Development of behavior patterns and myelinization of the nervous system in the human fetus and man. Contributions to Embryology. Monograph No. 139, Vol. XXIV. Washington, D. C.: Carnegie Institution.

ICHIJI TASAKI and FRANK D. WALKER

1. Own Relevant Publications

Hild, W., and Tasaki, I. (1962): Morphological and physiological properties of neurons and glial cells in tissue culture. J. Neurophysiol. 25:277.

Hild, W., Chang, J. J., and Tasaki, I. (1958): Electrical responses of astrocytic glia from the mammalian central nervous system cultivated in vitro. Experientia 14:220.

Tasaki, I., and Chang, J. J. (1958): Electric response of glial cells in cat brain. Science 128:1209.

H. L. WILLIAMS

1. Own Relevant Publications

Williams, H. L., Lubin, A., and Gieseking, C. F. (1959): Direct measurement of cognitive deficiency in brain-injured patients. J. Consult. Psychol. 23:300-305.

2. Other Suggested Background References

Parsons, O. A., Stewart, K. D., and Arenberg, D. (1957): Impairment of abstracting ability in multiple sclerosis. J. Nerv. Ment. Dis. 125:221-225.

B. Literature Cited At Work Session

1. Araki, T., and Terzuolo, C. A. (1962): Membrane currents in spinal motoneurons associated with the action potential and synaptic activity. J. Neurophysiol. 25:772.

2. Baldwin, Marcella V. (1952): A clinico-experimental investigation into the psychological aspects of multiple sclerosis. J. Nerv. Ment. Dis. 115:299.

3. Bodian, D. (1964): An electron microscopic study of monkey spinal cord. Bull. Johns Hopkins Hosp. 114:13.

4. Brinley, F. J., Jr., Kandel, E. R., and Marshall, W. H. (1960): Potassium outflux from rabbit cortex during spreading depression. J. Neurophysiol. 23:246.

5. Cammermeyer, J. (1960): Reappraisal of the perivascular distribution of oligodendrocytes. Amer. J. Anat. 106:197.

6. Canter, A. H. (1951): Direct and indirect measures of psychological deficit in multiple sclerosis, part I. J. Gen. Psychol. 44:3-25.

7. Diers, W. C., and Brown, C. C. (1950): Psychometric patterns associated with multiple sclerosis. Arch. Neurol. Psychiat. 63:760.

8. Dodt, E., and Jacobson, M. (1963): Photosensitivity of a localized region of the frog diencephalon. J. Neurophysiol. 26:752-758.

9. Flechsig, P. (1876): Die Leitungsbahnen im Gehirn und Rückenmark des Menschen. Leipzig: Engelmann.

10. Harreveld, A. van (1957): Changes in volume of cortical neuronal elements during asphyxiation. Amer. J. Physiol. 191:233-242.

11. Harreveld, A. van (1964): Extracellular space in central nervous tissue. Fed. Proc. 23:304.

12. Harrower, M. R., and Kraus, J. (1951): Psychological studies on patients with multiple sclerosis. A.M.A. Arch. Neurol. Psychiat. 66:44-57.

13. Hawkins, A., and Olszewski, J. (1957): Glia/Nerve cell index for cortex of the whale. Science 126:16-77.

14. Hess, H. H., and Pope, A. (1960): Intralaminar distribution of cytochromoxidase in human frontal isocortex. J. Neurochem. 5:207-217.

15. Hild, W., and Tasaki, I. (1962): Morphological and physiological properties of neurons and glial cells in tissue culture. J. Neurophysiol. 25:277.

16. Hortega, P. del Río (1928): Tercera apórtacion al conocimiento morfólogico e interpretación functional de la oligodendroglía. Mem. r. Soc. Españ. hist. nat. 14:5-122.

17. Kies, Marion W. (1964): Conference on Research in Demyelinating Disease. In: Galambos, R. G. (1964): Research in demyelinating diseases. NRP Bulletin II(2):4.

18. Korey, S. R., and Orchen, M. (1959): Relative respiration of neuronal and glial cells. J. Neurochem. 3: 277-285.

19. Kuhlenkampf, H. (1952): Verhalten der Neuroglia in den Vorderhörnern des Rückenmarkes der weissen Maus unter dem Reiz physiolopischen Tatigkeit. Z. Anat. Entw. 116:304-312.

20. Kupfer, C., and Palmer, P. (1964): Lateral geniculate nucleus: histological and cytochemical changes following afferent denervation and visual deprivation. Exp. Neurol. 9:400-409.

21. Loos, H. van der (1962): A substrate for ephaptic action of neurons in the cerebral cortex? In: Proceedings of the XXII International Congress of Physiological Sciences. (Leiden) Abstract no. 110.

22. Lowry, O. H., et al. (1954): The quantitative histochemistry of brain. J. Biol. Chem. 207:39-49.

23. Matthews, M. R. (1964): Further observations on transneuronal degeneration in the lateral geniculate nucleus of the macaque monkey. J. Anat. 98:255-264.

24. Pope, A. (1958): Implications of histochemical studies for metabolism of the neuroglia. In: Biology of Neuroglia. Windle, W. F., ed. Springfield, Ill.: C. C. Thomas. pp. 211-222.

25. Reed, D. J., Woodbury, D. M., and Holtzer, R. L. (1964): Brain edema, electrolytes and skeletal muscle. Arch. Neurol. 10:604-616.

26. Robins, E., and Smith, D. E. (1953): A quantitative histochemical study of eight enzymes of the cerebella-cortex and subjacent white matter in the monkey. Res. Publ. Ass. Res. Nerv. Ment. Dis. 305-327.

27. Sholl, D. A. (1956): The Organization of the Cerebral Cortex. New York: Wiley.

28. Svaetechin, G., Fatehchand, R., Laufer, M., Witkovsky, P., Negishi, K., and Selvin de Testa, A. (1963): Interacción glia-neuronal: Su depencia metabolica. Un huera teoria acerca del systema nervosa. Acta Cientifica Venezolana 14: Suppl. 1, 135-153.

29. Torack, R. M., Terry, R. D., and Zimmerman, H. M. (1959): The fine structure of cerebral fluid accumulation. I. Swelling secondary to cold injury. Amer. J. Pathol. 35:1135-1147.

30. Torack, R. M., Terry, R. D., and Zimmerman, H. M. (1959): The fine structure of cerebral fluid accumulation. II. Swellings produced by triethyl tin poisoning and its comparison with that in the human brain. Amer. J. Pathol. 36:273-287.

31. Tschirgi, R. D. (1958): The blood-brain barrier. In: Biology of Neuroglia. Windle, W. F., ed. Springfield, Ill.: C. C. Thomas. pp. 130-138.

32. Wardell, W. M. (1963): The response to electrical stimulation of neuroglial cells grown in tissue culture. J. Physiol. 169:25-26P.

33. Weiss, P., and Hiscoe, H. B. (1948): Experiments on the mechanism of nerve growth. J. Exp. Zool. 107:315.

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N E W S A N D V I E W S

From JAMES V. McCONNELL, Professor of Psychology, Mental Health Research Institute, The University of Michigan, Ann Arbor, Michigan:

"...In the July-August (1964) issue of the NRP Bulletin you published an article entitled "Failure to Train Planarians Reliably" by E. L. Bennett and M. Calvin. Since I believe that these authors have somewhat misinterpreted much of their own data, and because I believe that the picture of learning in planarians that they present is somewhat distorted, I have written a rebuttal entitled "Failure to Interpret Planarian Data Correctly: A Reply to Bennett and Calvin" which will be published in the next issue of the Worm Runner's Digest (due out about April 1, 1965). Naturally, I have sent a copy of my paper to Bennett and Calvin and will offer them equal space in the Digest if they care to comment on my rebuttal. I would greatly appreciate your calling this matter to the attention of all of your readers, who may obtain a copy of the pertinent issue of the Digest by writing to me. Sincerely,...

Two postdoctoral fellowships in neurophysiology are being offered by the Carnegie Institution of Washington for research in the autonomics division of England's National Physical Laboratory. Applicants must be U. S. citizens who have recently received their doctoral degree. The stipend will be $12,000 a year. The deadline for receipt of applications is April 1. Write to A. UTTLEY, Autonomics Division, National Physical Laboratory, Teddington, Middlesex, England.

A Summer Colloquium of Theoretical Biology will be held this year at Colorado State University. It will be under the administration of the American Institute of Biological Sciences, which will meet from July 1 to August 6, 1965. The program is sponsored by the National Aeronautic and Space Administration and is designed to stimulate and encourage active research in all areas of theoretical biology. The course of study will include lectures and seminars, but emphasis will be given to encouraging participants to carry out independent research. Both Pre- and Post-doctoral fellowships are available for individuals who wish to attend. Housing at the University is available to attendees and their families. For further information, write to the Director of the Institute, HAROLD J. MOROWITZ, Department of Molecular Biology and Biophysics, Box 2166 Yale Station, New Haven, Connecticut.

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